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Molecular identification and epidemiological data of *Anisakis* spp. (Nematoda: Anisakidae) larvae from Southeastern Pacific Ocean off Peru

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ABSTRACT

The objective of this study is to determine the infection status of nematode larvae and record epidemiological molecular data in commercial fish from the southeast Pacific off the central coast of Peru. Anisakiasis is a fish-borne zoonosis caused by *Anisakis* larvae, parasites of relevance in the fishery resources that have negative impact on public health. Between January 2012 to December 2014, 345 specimens of four fish species (*Trachurus symmetricus murphyi*, *Scomber japonicus peruanus*, *Merluccius gayi peruanus* and *Seriolella violacea*) were examined for *Anisakis* sp. larvae. A total of 997 *Anisakis* sp. larvae were found in the body cavity of 196 fish (total prevalence 53.7%, total mean intensity 5.08). After morphological analysis, 958 (96.08%) larvae were identified as Type I and 39 (3.92%) as Type II. Specimens were identified by molecular analysis of the mitochondrial cytochrome *c* oxidase subunit II (*cox*2) gene, confirming that *A. pegreffii* is the predominant species and the most important agent of human anisakiasis off the Peru Central Coast. In addition, we revealed the occurrence of *A. physeteris* (s.l.) in *S. japonicus peruanus* (P = 18.0%; MI = 2.17).

Therefore, the results obtained in the present study improve the knowledge of the occurrence of *Anisakis* species in the commercial fish from the Southeastern Pacific Ocean, highlighting the importance of considering a potential hazard for humans and the necessity of further research in other fishes of greater preference by the Peruvian population.

1. Introduction

Infections by fish-borne nematode larvae (L_3) affect human health worldwide, particularly in some countries such as Peru, Chile, Ecuador, or Colombia has been associated to the consumption of traditional raw fish-based dishes, such as ceviche, or insufficiently undercooked marinate (Cabrera and Suárez-Ognio, 2000; Cabrera and Trillo-Altamirano, 2004; Eiras et al., 2018; Martínez-Rojas et al., 2020). Despite the high consumption of raw fish in South America to date, few human cases have been reported, especially in Peru and Chile (Tantaleán and Huiza, 1993; Mercado et al., 1997, 2001, 2006; Barriga et al., 1999; Cabrera et al., 2003; Patiño and Olivera, 2019). Anisakiasis is a serious zoonosis produced by nematode larvae of the genus *Anisakis* that are widespread in fish populations worldwide acquiring a high social relevance for causing digestive disorders or initiating hypersensitivity states and allergies (Mattiucci et al., 2013, 2018). Dead worms can also cause allergic reactions and in the worst case leading to anaphylactic shock (Audicana et al., 2002). Fishes and cephalopods are paratenic hosts for the *Anisakis* larvae, while adult's parasites are found in marine mammals and seabirds, however, humans can become part of the cycle as accidental hosts (Mattiucci and Nascetti, 2008; Mattiucci et al., 2018). The European Food Safety Authority recommends that it is necessary to continue with the investigation of parasites present in fishery products implicated in public health (European Food Safety Authority EFSA, 2010). The "Organismo Nacional de Sanidad Pesquera" (SANIPES) is implementing normative for the protection against pathogens in fishery products to

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prevent the spread of diseases in Peru, being one of its priorities the control of zoosanitary parasites in hidrobiologic resources (SANIPES, 2020). For this reason, it is necessary to take preventive measures due to the increase of Peruvian fishery exports that represent about 336,942.98 tons of frozen hidrobiologic resources to countries like Spain (23%), China (14%), South Korea (12%), United States (8%), Thailand (6%), Italy (4%) and others (33%) (PRODUCE, 2018).

Larvae of *Anisakis* attached to the gastric mucosa extracted by endoscopy and expelled orally have been reported (Salazar and Barriga, 1999). During the El Niño Phenomenon 1997–1998, possible cases of anisakid infestation were recorded identifying the etiological agent of the larval stages this provides limited knowledge of the distribution and epidemiology of anisakid species distributed along the Southeastern Pacific Ocean (Cabrera and Suárez-Ognio, 2000; Mattiucci et al., 2018).

In the present study we provided epidemiology data, taxonomic and molecular identification of *Anisakis* spp. collected from four commercially important fish species according to the Encuesta Nacional de Hogares (Encuesta Nacional de Hogares ENAHO, 2018), where the consumption of fish by Peruvian families increased steadily in the last five years, going from 12.9 kilos per inhabitant in 2013 14.5 kilos in 2017.

2. Materials and methods

2.1. Sample collection

Between January 2012 and December 2014, 365 specimens of four commercial fish species were examined, *Trachurus symmetricus murphyi* (length 34.83 ± 2.32 cm; weight: 325.78 ± 54.7 g), *Merlucius gayi peruanus* (length 39.17 ± 2.6 cm; weight 459.2 ± 45.2 g), *Scomber japonicus peruanus* (length: 33 ± 2.7 cm; 315.5 ± 34.15 g) and *Seriorella violacea* (length 41 ± to 3.9 cm; weight 3895 ± to 85.4 g) off the coast of the Peruvian Sea (Lima and constitutional province of Callao) (Table 1).

Specimens were examined in fresh conditions in the laboratory of Parasitology in Wildlife and Zoonosis of "Universidad Nacional Mayor de San Marcos", fishes were identified according to Chirichigno and Cornejo (2001).

2.2. Parasitological examination

A total of 997 larvae were located in the body cavity, removed and repeatedly washed in 0.9% saline solution and morphologically identified at genus level by optical microscope (Leica EZ4, Germany). *Anisakis* larvae were grouped into Type I and II (sensu Berland, 1961), were stored in 2 ml tubes with 70% ethanol for molecular analysis, some larvae were fixed in 2.5% glutaraldehyde to be analyzed under the Scanning Electron Microscope (SEM).

2.3. Molecular identification

DNA was extracted with the DNeasy tissue Kit (Qiagen, Chatsworth, California, USA), DNA quality and quantity was checked in a spectrophotometer Nanodrop®ND-2000 (Thermo Scientific). The primers used were 211F (5′-TTT TCT AGT TAT ATA GAT TGR TTY AT-3′) and 210R (5′- CAC CAA ATC TTA AAA TTA TC-3′) (Valentini et al., 2006; Mattiucci et al., 2014). The locus was amplified by PCR in a Veriti™ 96-well thermocycler (Applied Biosystems, California, USA) with a final volume of 50 μL, including 5 μL of genomic DNA. The reaction mixture contained 2.5 U/μl Taq polymerase (Hot Star Taq DNA Polymerase Qiagen Kit, Hilden, Germany), and 0.5 μM of each primer (Macrogen, South

Table 1
Record of the total number, body length and weight of fish examined in the Southeastern Pacific Ocean off Peru during 2012–2014. Prevalence, mean intensity and mean abundance of *A. pegreffii* larvae.

Species	Parameter	Total	2012	2013	2014
Trachurus symmetricus murphyi	N	105	40	30	35
	Length ±SD	34.83 ± 2.32	34.5 ± 2.29	35.5 ± 2.3	34.5 ± 2.2
	Weight ±SD	325.78 ± 54.71	318.5 ± 52.71	341.93 ± 56.28	319.98 ± 52.08
	Prevalence	64.76	62.5	70	62.86
	CI 95%	55.47–74.05	46.82–78.18	52.60–87.40	46.02–79.70
	Mean intensity (range)	4.77 (1–10)	4.72 (1–10)	4.67 (1–9)	4.95 (2–10)
	CI 95%	4.14–5.42	3.67–5.77	3.43–5.91	3.70–6.21
	Mean abundance	3.1	2.95	3.27	3.11
	CI 95%	2.49–3.70	1.97–3.93	2.09–4.44	1.98–4.25
Merluccius gayi peruanus	**N**	**85**	**28**	**32**	**25**
	Length ± SD	39.17 ± 2.64	38.5 ± 2.17	39.5 ± 1.87	39.3 ± 2.59
	Weight ± SD	459.17 ± 95.28	431.02 ± 73.98	465.09 ± 67.51	459.17 ± 92.59
	Prevalence	77.65	78.57	75	80
	CI 95%	68.61–86.69	62.37–94.77	59.14–90.86	63.15–96.85
	Mean intensity (range)	3.7 (1–7)	3.86 (1–7)	3.83 (1–7)	3.35 (2–7)
	CI 95%	3.27–4.12	3.03–4.70	3.10–4.57	2.62–4.08
	Mean abundance	2.87	3.04	2.88	2.68
	CI 95%	2.40–3.34	2.14–3.93	2.06–3.69	1.87–3.49
Seriolella violacea	**N**	**75**	**26**	**24**	**25**
	Length ± SD	41 ± 3.89	39 ± 2.58	41.5 ± 2.87	42.5 ± 2.67
	Weight ± SD	3895 ± 369.97	3705 ± 245.29	3942.5 ± 272.87	4037.5 ± 271.96
	Prevalence	22.67	26.92	16.67	24
	CI 95%	12.97–32.36	8.65–45.19	0.59–32.74	6–41.99
	Mean intensity (range)	9.88 (1–48)	6 (1–10)	8.5 (7–10)	15.33 (7–48)
	CI 95%	4.66–15.11	2.89–9.11	6.45–10.55	(−)1.51–32.17
	Mean abundance	2.24	1.62	1.42	0
	CI 95%	0.79–3.69	0.33–2.90	0.04–2.80	(−) 0.41–7.78
Scomber japonicus peruanus	N	100	37	33	30
	Length ± SD	33 ± 2.74	32.5 ± 2.31	32.5 ± 1.71	33.5 ± 2.05
	Weight ± SD	315.58 ± 84.15	309.18 ± 62.42	308.68 ± 46.46	336.98 ± 64.81
	Prevalence	45	48.65	45.45	40
	CI 95%	35.08–54.92	31.75–65.54	27.52–63.38	21.39–58.61
	Mean intensity (range)	4.91 (1–9)	4.72 (1–9)	4.87 (2–8)	5.25 (2–9)
	CI 95%	4.26–5.56	3.53–5.91	3.96–5.78	3.64–6.86
	Mean abundance	2.21	2.3	2.21	2.1
	CI 95%	1.64–2.78	1.33–3.27	1.26–3.17	0.96–3.24

Korea). The amplification condition was optimized as follows: one cycle at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 46 °C for 60 s, 70 °C for 90 s, and a final cycle of 70 °C for 10 min; storage at 4 °C. The amplified fragments were visualized on 1% agarose gel.

The nucleotide sequences obtained by PCR were subjected to known sequences by BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the *Anisakis* spp. identified have been deposited in GenBank databases (accession numbers are highlighted in black, see Fig. 2), likewise, we use reference sequences obtained in other previous studies for the same gene and deposited in GenBank: *A. pegreffii* (JQ900759; JQ900760; JQ900761; MW074865; MW074866), *A. physeteris* (LCS43824; LCS43844; LCS43849; MW691145; MW691146; MW074867; MW074868; AB592798; DQ116432), *A. brevispiculata* (KC342901; KC342899), *A. paggiae* (KC821730; KC342896), *A. berlandi* (KC809999; KC810000), *A. simplex* s.str. (KC810003), A. typical (KF356650; KF356649), *A. ziphidarum* (KC821732, KC821736).

2.4. Phylogenetic analysis

Phylogenetic relationships were evaluated with maximum likelihood (ML) in the MEGA version X program (Kumar et al., 2018) using the Kimura's 2-parameter substitution model and the nodal support values were calculated by running 1000 bootstrap replicates (Kimura, 1980). Bayesian inference criteria (BIC) were analyzed in the Bayesian Evolutionary Analysis program by Sampling Trees (BEAST) version 1.7 (Drummond et al., 2012). The BIC model selected was HKY + G + I running a chain of 10 million generations and sampling tree topologies every 10,000 generations and the burning fraction were set at 10%.

2.5. Statistical analysis

The epidemiological parameters were determined following Rózsa et al. (2000). Prevalence (P%), mean intensity (MI) and mean abundance (MA) were calculated using the software Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005). Sterne's exact test was used at 95% confidence limits for prevalence. To compare MI and MA, the bootstrap procedure was applied with 1000 replications at the 95% confidence interval. Comparison between the levels of infection of *Anisakis* spp. larvae were calculated by Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005) using Fisher's exact test or exact unconditional test (prevalence-depending on sample size) (Reiczigel et al., 2008) and bootstrap two-sample *t*-test (mean abundance). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Morphological analysis

All the larvae collected from *S. violacea, T. symmetricus murphyi* and *M. gayi peruanus* were morphologically characterized as *Anisakis* Type I, and in *S. japonicus peruanus* co-infected by larvae of *Anisakis* Type I and II (sensu Berland, 1961). SEM showed the presence of an irregularly distributed cuticle with shallow transverse striation and very fine longitudinal striations distributed parallel to the body in both types of larvae. A small mouth with an oral opening was observed, surrounded by three rudimentary labial protuberances: one dorsal and two subventral, including a penetrating triangular tooth that is ventral with respect to the mouth and behind the excretory pore. In type I, the tail end has a slightly curved cone-shaped mucron and in type II it ends in tip (Fig. 1).

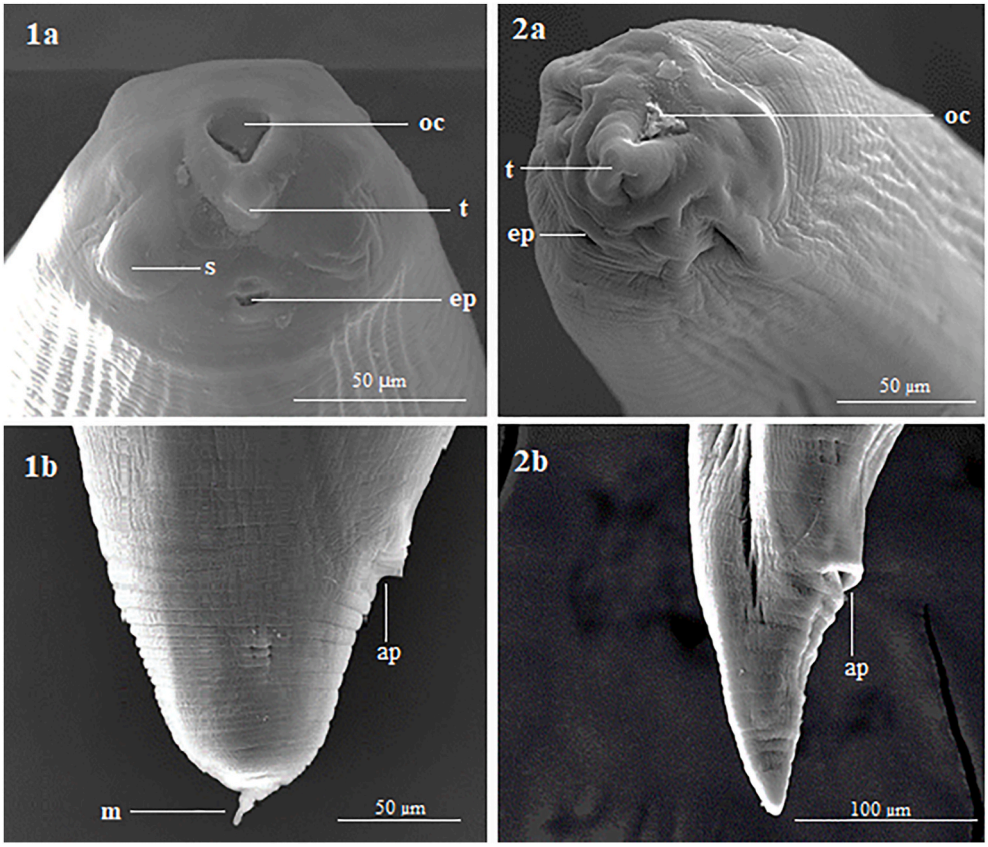

Fig. 1. Scanning electron micrographs of *Anisakis* type I and II.1a and 2a. Cephalic end.
Detail of the structures: oral cavity (oc), tooth (t), excretory pore (ep), subventral lip bulge (s). 1b. caudal end of *Anisakis pegreffii*. 2b. caudal end of *Anisakis physeteris*. Detail of the structures: anal pore (ap), mucron (m).

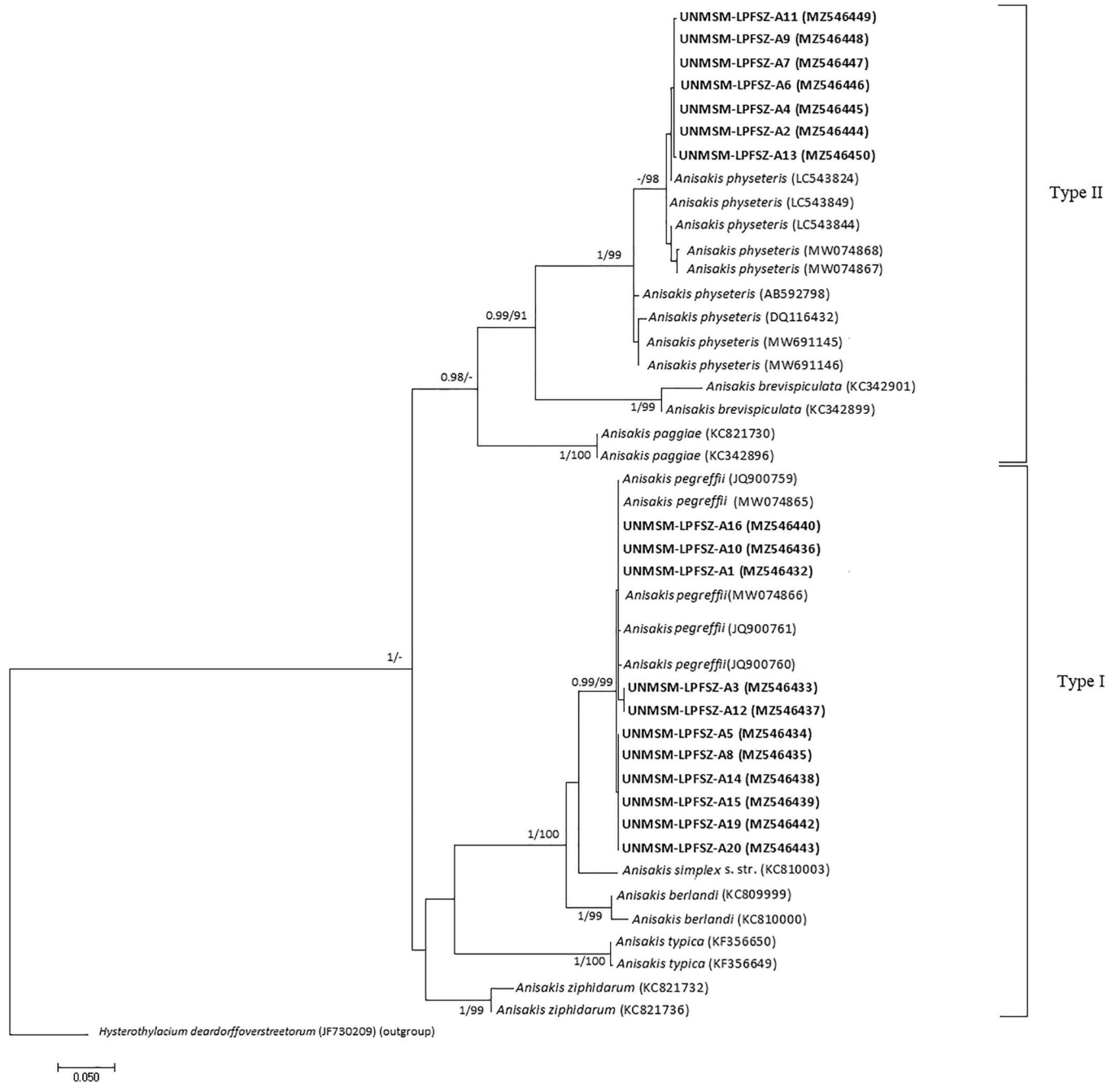

Fig. 2. Phylogenetic tree based on mtDNA *cox*2 gene sequences exploring the relationships among *Anisakis* species. The relationship was drawn using Bayesian inference (BI) and maximum likelihood (ML) methods. Posterior probability value (first) and nodal support is shown as bootstrap value (second) on the basis of 10 million generations for BI and 1000 replicates (only bootstrap values greater than 80% are shown) for ML, respectively. Scale bar indicate nucleotide substitutions per site. GenBank accession numbers are shown in parentheses. *Hysterothylacium deardorffoverstreetorum* was used as an outgroup.

3.2. Molecular identification and phylogenetic analysis

Amplification of the mitochondrial cytochrome *c* oxidase subunit II (*cox*2) gene of 19 larvae produced a fragment of about 600 bp. The results showed that *Anisakis* type I (N = 12) belongs to *A. pegreffii* (identity values of 99–100%) when comparing with the reference sequences deposited in GenBank (i.e., JQ900759; JQ900760; JQ900761; MW074865; MW074866). The two phylogenetic methods yielded the same results in terms of clades with high support values. However, the sequence obtained in the *cox*2 gene locus of *Anisakis* type II (N = 7) mtDNA is highly similar (identity values of 99.81–98.87%) from reference sequences recently deposited in GenBank (i.e., LC543824; LC543849; LC543844; MW074868; MW074867) of *A. physeteris*, but compared to other *cox2* mtDNA genetic sequences (e.i., AB592798; DQ116432; MW691146; MW691145) the genetic similarity was quite different (96.62%). The phylogenetic tree of the mtDNA *cox*2 gene was constructed using BI and ML confirming the assignment of type II larvae clustered with *A. physeteris* (s.l.) (Fig. 2).

3.3. Epidemiological parameters

A total of 997 *Anisakis* sp. larvae were found in 196 fish (total prevalence 53.7%, total mean intensity 5.08). After morphological analysis, 958 (96.08%) larvae were identified as type I and 39 (3.92%)

as type II. The hosts examined during the periods (2012–2014), *A. pegreffii* had a significantly higher prevalence in *T. symmetricus murphyi* (P = 64.76%), *M. gayus peruanus* (P = 77.65%), *S. japonicus peruanus* (P = 45%) compared to *S. violacea* (P = 22.67%).

The intensity of infection was significantly higher in *S. violacea* (MI = 9.88) compared to the other hosts. *A. pegreffii* and *A. physeteris* coexisted only in the pelagic species *S. japonicus peruanus*, where *A. pegreffii* (221/260) larvae had a significantly higher and constant intensity of infection that occurred in the study periods. Significant differences were found in abundance values through sampling years in the different hosts. The mean abundance in *T. symmetricus murphyi* (MA = 3.1) was significantly higher than in the other hosts. A constant mean abundance is observed in the sampled periods, except for *S. violacea*, which presented a significantly high difference in 2014 (Tables 1 and 2).

4. Discussion

In this study, we reported the presence of larvae of the *Anisakis* genus in the *Trachurus symmetricus murphyi, Scomber japonicus peruanus, Merluccius gayi peruanus* and *Seriolella violacea* caught in the Pacific Ocean off Peru. A high prevalence of *A. pegreffii* larvae was observed in the four fish species sampled. Between the years 2012–2014, no significant variations were found in the compared epidemiological parameters, however, the mean intensity varied significantly in *S. violacea*. During 2017 to 2018, Aco Alburqueque et al., 2020 reported a significant lower prevalence in *T. muphyi* and *S. japonicus* for the same geographic area of the present study. Furthermore, the parasitic loads found for *A. pegreffii* and *A. physteris* (s.l.) were consistent with our data. The hosts examined had commercial standard ranges (lenght and weight), where we observed that the largest fish showed a greater abundance of *Anisakis*. This correlation between the lenght of the fish and the parasite load is consistent with the information recorded by Aco Alburqueque et al., 2020 for the central Peruvian coast. In this area, recently *T. murphyi* and *S. japonicus* were coinfected with *A. pegreffii* and *A. physeteris* s.l. (Aco Alburqueque et al., 2020), however, during the years examined, we only reported coinfection in *S. japonicus*. The presence of *Anisakis* type II larvae was reported with a prevalence of 33% isolated from the intestine and mesentery of *Mugil cephalus* in the Colombian Pacific region (Castellanos et al., 2017), likewise, we reported *Anisakis* type II (*A. physeteris* s.l.) in *S. japonicus peruanus* with a prevalence of 18% in the central region of the Peruvian Pacific. In addition, *A. physeteris* (s.l.) larvae were reported on the north coast of Peru in jack mackerel, chub mackerel and Pacific bonito with very low prevalence (Aco Alburqueque et al., 2020). *Anisakis* species have been reported in cephalopods and marine fish of great importance for human consumption with a total prevalence of 26%–76.1% that depends on the geographical area of the host and season of the year (Mladineo et al., 2012; Chen and Shih, 2015; Cipriani et al., 2018; Molina-Fernández et al., 2018; Debenedetti et al., 2019). In the present study, we found a high prevalence of *Anisakis* type I (probably mostly *A. pegreffii*) in the same geographic area and fish species reported by Aco Alburqueque et al., 2020, in addition, we incorporated *M. gayi peruanus* as a new host infected by *A. pegreffii* larvae. High levels of infection of *A. pregreffii* larvae in European hake *Merluccius merluccius* were detected with 81.2% found in the viscera and liver, likewise, a positive correlation between fish length and abundance of *A. pegreffii* were observed (Cipriani et al., 2018). In the present study, the hakes from the Peruvian Sea infected by *A. pegreffii* larvae were isolated from the viscera and showed a similarity in the epidemiological parameters with the other host fish species (Table 1). *A. simplex* (s.s.) and *A. pegreffii* are the species most frequently found in the edible parts of commercially important fish and squid. Existing epidemiology data clearly indicate that not a single variable can explain alone the differences observed in infection by different *Anisakis* spp. in wild fisheries worldwide (Mattiucci et al., 2018). In fact, a recent study in squid of the genus *Histioteuthis* located in waters of the Central Mediterranean Sea were identified *A. physeteris* larvae present in the mantle (Palomba et al., 2021). Likewise, in the giant squid *Dosidicus gigas* of the Peruvian sea have being reported the presence of *Anisakis* type I and II (Céspedes et al., 2011).

The aim of the study was to contribute to the epidemiology and molecular identification of *Anisakis* spp. in commercially important fish from the Peruvian Sea. Despite the extensive presence of anisakid nematodes in fishery resources of the Peruvian Sea, there are few investigation of the levels of infection by *Anisakis* spp., as well as molecular and epidemiological data, furthermore, the etiological agent has not been identified at the species level (Céspedes et al., 2011; Chero et al., 2016; Serrano-Martínez et al., 2017; Martínez-Rojas et al., 2020).

Currently, PCR-DNA markers are the most used to study phylogenetic relationships between related anisakids, and have so far confirmed the existence of the sibling species previously detected by allozymes, establishing their taxonomic status (Mattiucci and Nascetti, 2008). The genetic/molecular markers based on ITS-rDNA and three mitochondrial genes (ie mtDNA *cox*1, ssrRNA and lsrDNA) were not able to disclose the two Antarctic sibling species of the *Contracaecum osculatum* (s.l.) complex. Instead, it makes be possible to distinguishing them not only by allozymes but also by the mtDNA *cox*2 gene locus (Mattiucci et al., 2008). Our morphological results determined the existence of two clades of *Anisakis*, therefore, we infered the phylogenetic analysis based on sequences of the *cox*2 mtDNA gene. Mitochondrial genes have shown high nucleotide genetic variation in nematode populations (Mattiucci and Nascetti, 2008), futhermore, various investigations mention that mtDNA *cox*2 gen has represented a valuable genetic marker for the analysis of the population structure of *Anisakis* spp. (Blažekovic et al., 2015; Mattiucci et al., 2017).

Aco Alburqueque et al., 2020 provided the first consistent molecular data through *cox*2 mtDNA sequence analysis and identified *A. pegreffii* for all *Anisakis* type I larvae, however, they detected a new gene pool for *Anisakis* type II larvae in the *A. physeteris* (s.l.) species complex. From the inferred analyzes of the BI and ML trees derived from mtDNA *cox*2 gene nucleotide sequence, types I and II larvae belonged to the clades of *A. pegreffii* and *A. physeteris* (s.l.), respectively. The molecular results confirmed that in the central Peruvian Sea, the fish species studied were infected with *Anisakis* species most reported globally.

The occurrence of *A. physeteris* has been reported in different Mediterranean fish but with low prevalence (Brogli and Kapel, 2011; Lim et al., 2015; Cipriani et al., 2016, 2018), however, high infection values were found in the gonads in squid species (Palomba et al., 2021). For the South Pacific Ocean, a low prevalence of *A. physeteris* (s.l.) has been reported (Aco Alburqueque et al., 2020), likewise, we reported low levels of infection of *A. physeteris* (s.l.) larvae in the sampled area of the

Table 2
Prevalence, mean intensity and mean abundance of *A. physeteris* larvae in sampled of *S. japonicus peruanus* during 2012–2014.

	Parameter	Total	2012	2013	2014
Scomber japonicus peruanus	Prevalence	18	13.51	18.18	23.33
	CI 95%	10.34–25.66	1.96–25.07	4.29–32.07	7.27–39.40
	Mean intensity (range)	2.17 (1–3)	2.4 (2–3)	2.17 (1–3)	2 (1–3)
	CI 95%	1.78–2.56	1.72–3.08	1.38–2.96	1.08–2.92
	Mean abundance	0.39	0.32	0.39	0.47
	CI 95%	0.21–0.57	0.04–0.61	0.08–0.71	0.10–0.83

central coast Peruvian. Additionally, the five sequences obtained have 99% identity to the sequences previously deposited in GenBank by Aco Alburqueque et al., 2020. This would represent a new gene pool in the *A. physeteris* (s.l.) species complex, and needs further genetic investigation of other gene loci to clarify their phylogenetic status and taxonomic position as also suggested in the findings by Aco Alburqueque et al., 2020. *A. pegreffii* is common in fish from the Mediterranean and Adriatic Sea and has also been reported as a common and dominant species in different fish species (Mladineo et al., 2014; Molina--Fernández et al., 2018; Cipriani et al., 2018; Debenedetti et al., 2019). Furthermore, the levels of infection by *A. pegreffii* can oscillate significantly according to the geographical area, as recorded in *Merluccius merluccius* from the Tyrrhenian Sea and the Spanish Atlantic coast (Cipriani et al., 2015). In the Southeastern Pacific Ocean off the Peru coast, a high prevalence of *A. pegreffii* has been reported (Aco Alburqueque et al., 2020). Similarly, we reported a significantly high incidence and prevalence of *A. pegreffii* in fish hosts sampled in the central Peruvian Sea. In addition, a study revealed pronounced differences in the level and pattern of infection of the *Anisakis* species among mackerel populations, where *A. pregreffii* was the dominant species in Mediterranean waters sample locations, while *A. simplex* (s.s.) was the most species prevalent in mackerel samples from Atlantic catching areas (Levsen et al., 2018). We reported mixed infections with species of *A. pegreffii* and *A. physeteris* (s.l.) in samples of Peruvian mackerel, being *A. pregreffii* highly predominant, these results agree with the study by Aco Alburqueque et al., 2020 for two capture areas of mackerel samples from the Peruvian sea.

Anisakiasis has been considered an emerging zoonosis less than a decade ago in Italy (Mattiucci et al., 2013), Korea (Lim et al., 2015) and Croatia (Brogli and Kapel, 2011), being *A. pegreffii*, the most important ethiological agent of human anisakiasis. In Japan, reported 158 patients that manifested acute gastrointestinal discomfort caused by anisakiasis, where they recommend endoscopy for the diagnosis of suspected cases (Furuya et al., 2018).

In Poland, the first case of gastric anisakiasis due to *A. simplex* (s.s.) was reported, the larvae were removed alive from an adult woman who manifesting persistent stomach pain (Kołodziejczyk et al., 2020). To date, in several clinical case reports, the molecular diagnosis of the etiologic agent has been shown that only *A. simplex* (s.s.) and *A. pegreffii* have the ability to cause "invasive anisakiasis" in humans (Mattiucci et al., 2018). As consumption of fishery resources increases in Peruvian, reports of anisakiasis are likely to be more frequent, for this reason, it is necessary for health centers record cases of patients that present as symptoms gastrointestinal ailments after the consumption of fish and shellfish as possible clinical reports of anisakiasis. Therefore, prevention and protection against zoonotic parasites in fishery products intended for human consumption have become a priority (Levsen and Lunestad, 2010).

In the present study, the viscera and musculature were thoroughly examined, however, all the larvae were isolated from the coelomic cavity of teleost fish. Aco Alburqueque et al., 2020 reported the presence of *A. pegreffii* larvae in fish flesh isolated from *T. murphyi* and *S. japonicus* with very low prevalence for Northern and Central coast of Peru. So far, the behavior of *Anisakis* larvae is unknown, they generally remain in the visceral cavity or within the visceral organs of the fish, while, in other cases, they can migrate and penetrate deep into the muscle and are difficult to detect by visual inspection (Levsen and Lunestad, 2010; Cipriani et al., 2016; Levsen et al., 2017).

The risk management measures for *Anisakis* must be adapted to commercial species, considering all ecological and phylogenetic traits of the host-parasite found in a specific fishing ground will be encompassed, resulting in satisfactory control conditions (Mladineo and Poljak, 2014). These measures are being adapted and regulated under the standards of the European Community (CE) and the US Food and Drug Administration (FDA).

In conclusion, the reported results provide valuable information on the occurrence of *Anisakis* in the study area, suggesting that the dominant species on the central coast of the Peruvian Sea is *A. pegreffii*, it can be used to guide public policies in the fishing sector.

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.

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References

Aco Alburqueque, R., Palomba, M., Santoro, M., Mattiucci, S., 2020. Molecular identification of zoonotic parasites of the genus *Anisakis* (nematoda: anisakidae) from fish of the southeastern Pacific Ocean (off Peru coast). Pathogens 9 (11), 910.
Audicana, M.T., Ansotegui, I.J., de Corres, L.E., Kennedy, M.W., 2002. *Anisakis simplex*: dangerous – dead and alive? Trends Parasitol. 18, 20–25.
Barriga, J., Salazar, F., Barriga, E., 1999. Anisakiasis: presentación de un caso y revisión de la literatura. Rev. Gastroenterol Peru. 19, 317–323.
Brogli, A., Kapel, C., 2011. Changing dietary habits in a changing world: emerging drivers for the transmission of foodborne parasitic zoonoses. Vet. Parasitol. 182, 2–13.
Cabrera, R., Suárez-Ognio, L., 2000. Probable emergencia de anisakiosis por *Anisakis physeteris* durante el fenómeno El Niño 1997–1998 en la costa peruana. Parasitol. Latinoam. 57, 166–170.
Cabrera, R., Luna-Pineda, N., Suárez-Ognio, L., 2003. Nuevo caso de infección humana por una larva de *Pseudoterranova decipiens* (Nematoda, Anisakidae) en el Perú. Rev. Gastroenterol. Perú. 23, 217–220.
Cabrera, R., Trillo-Altamirano, M.D.P., 2004. Anisakidosis: ¿Una zoonosis parasitaria marina desconocida o emergente en el Perú? Rev. Gastroenterol. Peru. 24, 335–342.
Castellanos, J.A., Tangua, A.R., Salazar, L., 2017. Anisakidae nematodes isolated from the flathead grey mullet fish (*Mugil cephalus*) of Buenaventura, Colombia. Int J. Parasitol. Parasites Wildl. 6 (3), 265–270.
Céspedes, R.E., Iannacone, J., Salas, A., 2011. Helmintos parásitos de *Dosidicus gigas* "Pota" eviscerada en Arequipa. Perú. Ecol. Apl. 10, 1–11.
Chen, H.Y., Shih, H.H., 2015. Occurrence and prevalence of fish-borne *Anisakis* larvae in the spotted mackerel *Scomber australasicus* from Taiwanese waters. Acta Trop. 145, 61–67.
Chero, J., Sáez, G., Iannacone, J., Cruces, C., Alvariño, L., Luque, J., 2016. Ecología comunitaria de metazoos parásitos del bonito *Sarda chiliensis* Cuvier, 1832 (Perciformes: scombridae) de la costa peruana. Rev. Inv. Vet. Perú. 27 (3), 539–555.
Chirichigno, N., Cornejo, R.M., 2001. Catálogo comentado de los peces marinos del Perú. Callao. Instituto del Mar del Perú, Perú.
Cipriani, P., Smaldone, G., Acerra, V., D'Angelo, L., Anastasio, A., Bellisario, B., Palma, G., Nascetti, G., Mattiucci, S., 2015. Genetic identification and distribution of the parasitic larvae of *Anisakis pegreffii* and *Anisakis simplex* (s. s.) in European hake *Merluccius merluccius* from the Tyrrhenian Sea and Spanish Atlantic coast: implications for food safety. In. J. Food Microbiol. 198, 1–8.
Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G.L., Cheleschi, R., Nascetti, G., Mattiucci, S., 2016. Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: anisakidae) in European anchovy, *Engraulis encrasicolus*: implications to seafood safety. Food Contr. 59, 148–157.
Cipriani, P., Sbaraglia, G.L., Paoletti, M., Giulietti, L., Bellisario, B., Palomba, M., Bušelić, I., Mladineo, I., Nascetti, G., Mattiucci, S., 2018. The Mediterranean European hake, *Merluccius merluccius*: detecting drivers influencing the *Anisakis* spp. larvae distribution. Fish. Res. 202, 79–89.
Debenedetti, Á.L., Madrid, E., Trelis, M., Codes, F.J., Gil-Gómez, F., Sáez-Durán, S., Fuentes, M.V., 2019. Prevalence and risk of anisakid larvae in fresh fish frequently consumed in Spain: an Overview. Fishes 4 (1), 13.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Eiras, J.C., Pavanelli, G.C., Takemoto, R.M., Nawa, Y., 2018. Fish-borne nematodiases in South America: neglected emerging diseases. J. Helminthol. 92, 649–654.
Encuesta Nacional de Hogares (ENAHO), 2018. Instituto Nacional de Estadística e Informática – INEI, Lima.
European Food Safety Authority (EFSA), 2010. Scientific opinion of the Panel on Biological Hazards on risk assessment of parasites in fishery products. EFSA J 8, 10–43.
Furuya, K., Nakajima, H., Sasaki, Y., Urita, Y., 2018. Anisakiasis: the risks of seafood consumption. Niger. J. Clin. Pract. 21 (11), 1492–1494.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Kołodziejczyk, L., Szostakowska, B., Sobecka, E., Szczucki, K., Stankiewicz, K., 2020. First case of human anisakiasis in Poland. Parasitol. Int. 76, 102073.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. Mega X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

Levsen, A., Lunestad, B.T., 2010. *Anisakis* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. Vet. Parasitol. 171, 247–253.

Levsen, A., González, A.F., Mattiucci, S., Cipriani, P., Paoletti, M., Gay, M., Højgaard, D. P., Joensen, M.M., Hastie, L.C., Bao, M., MacKenzie, K., Pierce, G.J., Karl, H., Ostermeyer, U., Buchmann, K., Bušelić, I., Mladineo, I., Pascual, S., 2017. A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—introducing the FP7 PARASITE exposure assessment study. Fish. Res. 202, 4–21.

Levsen, A., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L.C., MacKenzie, K., Pierce, G.J., Svanevik, C.S., Højgaard, D.P., Nascetti, G., González, A.F., Pascual, S., 2018. *Anisakis* species composition and infection characteristics in Atlantic mackerel, *Scomber scombrus*, from major European fishing grounds—reflecting changing fish host distribution and migration pattern. Fish. Res. 202, 112–121.

Lim, H., Jung, B.K., Cho, J., Yooyen, T., Shin, E.H., Chai, J.Y., 2015. Molecular diagnosis of cause of anisakiasis in humans, South Korea. Emerg. Infect. Dis. 21, 342–344.

Martínez-Rojas, R., Mondragón-Martínez, A., Pulido-Murillo, E.A., Tantalean-Vidaurre, M., 2020. Caracterización del patrón proteico y perfil antigénico de larvas L3 de anisakidos de importancia médica aisladas de peces comerciales de la costa peruana. An. Fac. Med. 81 (3), 324–329.

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.

Mattiucci, S., Paoletti, M., Webb, S.C., Sardella, N., Timi, J.T., Berland, B., Nascetti, G., 2008. Genetic relationships among species of *Contracaecum* Railliet and Henry, 1912 and *Phocascaris* Höst, 1932 (Nematoda: anisakidae) from pinnipeds based on mitochondrial cox2 sequences, and congruence with allozyme data. Parasite 15 (3).

Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Salomone 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.

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* sp. for *A. simplex* sp. C (Nematoda: anisakidae). J. Parasitol. 100, 199–214.

Mattiucci, S., Giulietti, L., Paoletti, M., Cipriani, P., Gay, M., Levsen, A., Klapper, R., Karl, H., Bao, M., Pierce, G.J., Nascetti, G., 2017. Population structure of the parasite *Anisakis simplex* (s. s.) collected in *Clupea harengus* L. from North East Atlantic fishing grounds. Fish. Res. 202, 103–111.

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. 99, 93–263.

Mercado, R., Torres, P., Maira, J., 1997. Human case of gastric infection by a fourth larval stage of *Pseudoterranova decipiens* (Nematoda, Anisakidae). Rev. Saude Publica 31 (2), 178–181.

Mercado, R., Torres, P., Muñoz, V., Apt, W., 2001. Human infection by *Pseudoterranova decipiens* (Nematoda, Anisakidae) in Chile report of seven cases. Mem. Inst. Oswaldo Cruz 96 (5), 653–655.

Mercado, P.R., Torres, H.P., Gil, L.L.C., Goldin, G.L., 2006. Anisakiasis en un paciente portador de una pequeña hernia hiatal. Caso clínico. Rev. Med. Chile 134 (12), 1562–1564.

Mladineo, I., Šimat, V., Miletić, J., Beck, R., Poljak, V., 2012. Molecular identification and population dynamic of *Anisakis pegreffii* (nematoda: anisakidae dujardin, 1845) isolated from the European anchovy (*Engraulis encrasicolus* L.) in the Adriatic Sea. Int. J. Food Microbiol. 157, 224–229.

Mladineo, I., Poljak, V., 2014. Ecology and genetic structure of zoonotic *Anisakis* spp. from Adriatic commercial fish species. Appl. Environ. Microbiol. 80 (4), 1281–1290.

Molina-Fernández, D., Rubio-Calvo, D., Adroher, F.J., Benítez, R., 2018. Molecular epidemiology of *Anisakis* spp. in blue whiting *Micromesistius poutassou* in eastern waters of Spain, western Mediterranean Sea. Int. J. Food Microbiol. 282, 49–56.

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 (1), 1–12.

Patiño, J.A., Olivera, M.J., 2019. Anisakiasis gastro-alérgica, primera descripción de un caso en Colombia y revisión bibliográfica. Biomedica 39 (2), 241–246.

PRODUCE, 2018. Anuario Estadístico Pesquero y Acuícola 2018. La actividad productiva del sector en números. Ministerio de la Producción, Perú.

Reiczigel, J., Rózsa, L., 2005. Quantitative Parasitology 3.0. Budapest. Distributed by the authors.

Reiczigel, J., Abonyi-Tóth, Z., Singer, J., 2008. An exact confidence set for two binomial proportions and exact unconditional confidence intervals for the difference and ratio of proportions. Comput. Stat. Data Anal. 52, 5046–5053.

Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. J. Parasitol. 86, 228–232.

Salazar, F., Barriga, E., 1999. Anisakiasis: presentación de un caso y revisión de la literatura. Rev. Gastroenterol. Perú. 19 (4), 317–323.

SANIPES, 2020. Informe de inocuidad 2017-2019. Ministerio de la Producción, Perú.

Serrano-Martínez, E., Quispe, M., Hinostroza, E., Plasencia, L., 2017. Detección de parásitos en peces marinos destinados al consumo humano en Lima Metropolitana. Rev. Inv. Vet. Perú 28 (1), 160–168.

Tantaleán, M., Huiza, A., 1993. Nematode larvae with medical importance found in sea fish of Peruvian shore, with two records of human infection. Rev. Per. Med. Trop. UNMSM. 7, 61–65.

Valentini, A., Mattiucci, S., Bondanelli, P., Webb, S.C., Mignucci-Giannone, A.A., Colom-Llavina, M.M., Nascetti, G., 2006. Genetic relationships among *Anisakis* species (Nematoda: anisakidae) inferred from mitochondrial cox-2 sequences, and comparison with allozyme data. J. Parasitol. 92, 156.