International Journal of Food Microbiology 267 (2018) 20–28

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Distribution of anisakid nematodes parasitizing rajiform skates under commercial exploitation in the Southwestern Atlantic

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ARTICLE INFO

Keywords:
Anisakis pegreffii
Anisakis berlandi
Pseudoterranova cattani
Sympterygia bonapartii
Atlantoraja castelanui
Zearaja chilensis
Argentine Sea

ABSTRACT

In order to evaluate the infestation by anisakids present in elasmobranchs and their distribution in the Argentine Sea, this study was carried at a regional scale with the following aims: 1) to identify those anisakid species present in skates under exploitation; 2) to characterize quantitatively these infestations and 3) to determine those factors driving the variability in parasite burdens across skate species. A total of 351 skates, belonging to 3 species (218 *Sympterygia bonapartii*, 86 *Zearaja chilensis* and 47 *Atlantoraja castelnaui*) and from different localities of the Argentine Sea were examined for anisakids. Parasites were found in the stomach wall at high prevalence in some samples. Based on morphology and mtDNA *cox2* sequences analyses (from 24 larval worms), specimens were identified as *Anisakis berlandi*, *A. pegreffii* and *Pseudoterranova cattani*; the last two known as potentially pathogenic for humans. Differential distribution patterns were observed across parasite and hosts species. In general, fish caught in southern and deeper waters exhibited higher loads of *Anisakis* spp., whereas infestation levels by *P. cattani* increase in larger skates. Taking into account that the mere presence of worms or their antigens in fish meat can provoke allergic responses, information on distribution of parasites and their variability is essential for the implementation of food safety practices.

1. Introduction

Nematodes of the family Anisakidae are cosmopolitan parasites of aquatic systems. Some of their representatives are known by their implication in human health as causative agents of anisakidosis, an inflammation of the gastrointestinal tract caused by the ingestion of raw or undercooked fish or squid containing third-stage larvae (Audicana and Kennedy, 2008; Mattiucci and Nascetti, 2008). Moreover, exposure to the parasites and their antigens/allergens, not only in the form of a living infestation, but also by consumption of dead parasites in food fish, is increasingly recognized as a widespread problem with many clinical manifestations in humans. These can be classified as gastric, intestinal, and ectopic anisakidosis and allergic forms (Audicana and Kennedy, 2008; Mattiucci et al., 2011, 2017a).

Among anisakids, the genus *Anisakis* and, to a lesser extent, the genus *Pseudoterranova* are known as the responsible of most human infestations (Mattiucci and Nascetti, 2008), accounting for the majority of about 20.000 cases reported worldwide since 1960s (Audicana and Kennedy, 2008; Hochberg and Hamer, 2010). *Anisakis* is composed of nine species (Mattiucci et al., 2014; Valentini et al., 2006) that differ in their host preferences, ecology and zoogeography (Gómez-Mateos et al., 2016; Mattiucci and Nascetti, 2008; Mattiucci et al., 2017b, 2017c). It has been proposed that differences also exist in their pathogenic potential (Arizono et al., 2012; Romero et al., 2013, 2014) and allergenic capacities (Arcos et al., 2014). *Pseudoterranova* comprises six species that parasitize pinnipeds. They belong to the *Pseudoterranova decipiens* complex and include the etiologic agents of anisakidosis (Mattiucci and Nascetti, 2008). As in the case of *Anisakis*, *Pseudoterranova* species also differ in their definitive hosts, ecology, zoogeography and pathogenicity to humans (Arizono et al., 2011; Desowitz, 1986; Mattiucci and Nascetti, 2008; McClelland, 2002; Timi et al., 2014).

This broad spectrum of variability sources in anisakid infestations, either for natural hosts or for humans, makes the knowledge of their geographical distribution, host range, and epidemiology a priority for the implementation of measures to prevent from and protect against these zoonotic parasites, considered one of the most significant

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https://doi.org/10.1016/j.ijfoodmicro.2017.12.009
Received 9 June 2017; Received in revised form 4 October 2017; Accepted 10 December 2017
Available online 19 December 2017

Table 1
Records of anisakid genera *Anisakis* and *Pseudoterranova* in elasmobranch (selacean and batoid) hosts in chronological order of publication (hosts nomenclature as published by the respective authors).

Source	Anisakid species	Microhabitat	Host/s	Locality
Selacean hosts				
Zhukov, 1960	*Anisakis* sp.	Ni	*Squalus acanthias*	Japan
Threlfall, 1969	*Anisakis* sp.	Ni	*S. acanthias*	Canada
	Pseudoterranova sp. (as *Porrocaecum* sp.)	Ni	*S. acanthias*	Canada
Hewitt and Hine, 1972	*Anisakis* sp.	St[a]	*Carcharodon carcharias*, *Cephaloscyllium isabella*, *Dalatias licha*, *Deania calcea*, *Galeorhinus australis*, *Isurus oxyrinchus*, *Notorynchus cepedianus*, *Prionace glauca*, *S. acanthias*.	New Zealand
Orlowska, 1979	*Anisakis simplex*	Bc	*S. acanthias*	North Sea
Torres et al., 1983	*Anisakis* sp.	Ni	*Schoroederichthys chilensis*	Chile
	Pseudoterranova sp. (as *Phocanema* sp.)	Ni	*S. chilensis*	Chile
Wierzbicka and Langowska, 1984	*Anisakis simplex*	Bc	*S. acanthias*	New Zealand
Fernández and Villalba, 1985	*Anisakis* sp. (Type I)	Ni	*Halaelurus canescens*, *Squalus fernandinus*	Chile
	Anisakis sp.	Ni	*Echinorhinus cookei*	Chile
Henderson and Dunne, 1998	*Anisakis simplex*	St serosa	*Scyliorhinus canicula*	Ireland
Knoff et al., 2001	*Anisakis* sp.	St, Sv	*Hexanchus griseus*, *Heptranchias perlo*, *Squalus megalops*, *Mustelus canis*, *Galeorhinus vitaminicus*, *Carcharhinus signatus*, *Squatina* sp.	Brazil
	Pseudoterranova sp.	St, Sv	*S. megalops*, *M. canis*, *Mustelus schmitti*, *G. vitaminicus*	Brazil
Moore, 2001	*Anisakis simplex*	St serosa	*S. canicula*	England and Wales
	Pseudoterranova decipiens	St and Sv serosa, Bc	*S. canicula*	England and Wales
Palm and Schröder, 2001	*Anisakis* sp. (Type I)	Ni	*Deania histricosa*	Central East Atlantic
	Anisakis sp. (Type II)	Ni	*Heptranchias perlo*, *D. histricosa*, *D. calcea*, *D. profundorum*	Central East Atlantic
Rokicki et al., 2001	*Anisakis simplex*	St and Sv serosa	*Raja radiate*, *R. hyperborea*, *Bathyraja spinicauda*	Norway
Henderson et al., 2002	*Anisakis simplex*	St and Sv serosa and lumen	*S. acanthias*	Ireland
Klimpel et al., 2003	*Anisakis simplex*	St serosa, Bc	*Etmopterus spinax*	Norway
Purivirojkul et al., 2009	*Anisakis* sp.	Sv	*Alopias pelagicus*, *Squalus mitsukurii*	Thailand
Kuhn et al., 2011	*Anisakis simplex s.s.*[b]	St and Sv serosa, BC[c]	*E. spinax*, *E. pusillus*, *E. princeps*, *D. profundorum*	Azores
	Anisakis physeteris[b]	St and Sv serosa, BC[c]	*E. spinax*	Azores
Costa et al., 2014	*Anisakis simplex s.s.*[b]	St lumen	*Centrophorus squamosus*	Madeira Archipelago
	Pseudoterranova ceticola[b]	St lumen	*C. squamosus*	Madeira Archipelago
Isbert et al., 2015	*Anisakis* sp. (Type I)	St, Sv, Bc	*E. spinax*	Spain
Gračan et al., 2016	*Anisakis pegreffii*[b]	St and Sv lumen	*Mustelus punctulatus*, *S. acanthias*	Adriatic Sea
Batoid hosts				
Threlfall, 1969	*Anisakis* sp.	Ni	*Raja radiata*	Canada
	Pseudoterranova sp. (as *Porrocaecum* sp.)	Ni	*R. radiata*	Canada
Hewitt and Hine, 1972	*Anisakis* sp.	St[a]	*Raja* sp., *Torpedo fairchildi*	New Zealand
McVicar, 1977	*Anisakis* sp.	St serosa	*Raja naevus*	Scotland
Fernández and Villalba, 1985	*Anisakis* sp.	Ni	*Raja chilensis*	Chile
	Anisakis sp. (Type I)	Ni	*Psammobatis caudispina*	Chile
	Pseudoterranova sp. (as *Phocanema* sp.)	Ni	*R. chilensis*	Chile
Knoff et al., 2001	*Anisakis* sp.	St, Sv	*Dipturus trachyderma*	Brazil
Álvarez et al., 2006	*Anisakis simplex*	St	*Raja microocellata*, *R. brachyura*	Spain
Moya et al., 2015	*Anisakis* sp.	Bc	*Atlantoraja platana*	Argentina

[a] Microhabitat reported by Wharton et al. (1999).
[b] Identification based on molecular tools.
[c] T. Kuhn pers. comm. Bc: body cavity; Ni: not indicated; St: stomach; Sv: spiral valve.

emerging food-borne zoonoses (McCarthy and Moore, 2000).

Due to the relevance of anisakids for human health, and also because of their significance on the commercial value of fish products, being a chronic and costly cosmetic problem for seafood processors (McClelland, 2002), a vast amount of literature has dealt with this group of parasites. A considerable proportion of these publications include worldwide reports of infestations with anisakids in fish, which are characterized by an increasing rate of molecular identifications and by an overwhelming majority of surveys on teleosteans over elasmobranchs. Indeed, it is often postulated that infestations with nematodes in the elasmobranchs body cavity and tissues are rare, especially for larval stages. This is said to be due to the high concentrations of urea, which makes elasmobranchs body an unfavourable environment for helminths (Caira and Healy, 2004; Moya et al., 2015). Nevertheless, reports on zoonotic anisakids in elasmobranchs are frequently found in the literature, as shown in Table 1. From these data, it is possible to make some generalizations: first, selaceans (sharks, dog-fishes, etc.) have more commonly been reported as hosts for anisakids than batoids (skates, rays); second, despite the fact that many of the records report the presence of worms in the gastrointestinal lumen, indicating that transience of the parasites in these hosts, a considerable number of papers recorded their presence in tissues or body cavity, and they can be considered as true parasites; third, the use of molecular techniques to unequivocally identify anisakids at specific level in elasmobranchs is

still incipient, however different species have been recorded infesting selaceans, but no data are available on parasites of batoids.

In the last years, available molecular approaches have promoted a great increase on the taxonomy, ecology, clinical and epidemiological relevance of anisakids (Kuhn et al., 2011, 2013; Mattiucci and Nascetti, 2008), and their zoogeography is being increasingly revealed at global scales (Kuhn et al., 2011, 2013; Shamsi, 2014; Shamsi et al., 2012). However, the potential risk of this zoonosis remains underestimated for some important fishery products, such as elasmobranchs, and for some regions, such as southwestern Atlantic. Indeed, the average declared value of total world imports of chondrichthyan meat was 123,960 tons per year between 2000 and 2011 (Dent and Clarke, 2015). In particular, the chondrichthyan catch in Argentine waters is the fifth at global level (Subsecretaría de Pesca y Acuicultura, 2016). As regards skates, they represent the 98% of the total chondrichthyan volume exported; being the 70% of it commercialized as fins, and the 30% as whole skates (Subsecretaría de Pesca y Acuicultura, 2016), however, no data are available on parasitism by anisakids in these products.

Therefore, the potential of elasmobranchs as sources of infestation of anisakidosis for humans requires an assessment. Particularly taking into account the recent worldwide increase in the demand of shark, skate and ray fins and meat mainly by Asian markets (Dent and Clarke, 2015), and the fact that skates have been recently identified as probable sources of infestation in humans (Sohn et al., 2015). Consequently, in order to assess and characterize the distribution of larval anisakids in skates from the southwestern Atlantic, the aim of this study is threefold: 1) to identify those anisakid species present in skates from southwestern Atlantic; 2) to characterize quantitatively these infestations in skate species under exploitation, and 3) to determine those factors driving the variability in parasite burdens across skate species and zones at a regional scale.

2. Materials and methods

2.1. Fish sampling and parasite inventories

A total of 351 skates were examined for anisakids, including 218 specimens of the smallnose fanskate *Sympterygia bonapartii* Müller and Henle, 1841, 86 of the yellownose skate *Zearaja chilensis* (Guichenot, 1848) and 47 of the spotback skate *Atlantoraja castelnaui* (Miranda Ribeiro, 1907). A detail of samples composition is given in Table 2. Most fish were caught during research cruises of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), covering the Argentine shelf and the Argentine-Uruguayan Common Fishing Zone, south of 34° S. Additional samples were obtained from commercial trawlers operating off the coast of Buenos Aires Province, Argentina (Fig. 1). Host identification was made following the key of Cousseau et al. (2007).

Samples from research cruises were kept frozen at −20 °C until examination, whereas those from commercial trawlers were examined in fresh condition. In all cases, fins, body cavity and viscera were examined under a stereomicroscope.

2.2. Nematode species identification

For occurrence and site recording purposes, *Anisakis* larvae were identified at genus level based on morphological criteria before subsequent molecular analyses. A subsample of 22 *Anisakis* spp. larvae, randomly selected from different hosts and localities, was prepared for analysis of the mitochondrial cytochrome *c* oxidase subunit 2 (mtDNA *cox2*) gene. Larval *Pseudoterranova* were identified to species level, based on their morphology/morphometry (Timi et al., 2014) and their identity was confirmed by genetic analyses of mtDNA *cox2* from 2 specimens.

2.3. DNA extraction, amplification and sequencing of the mtDNA cox2 gene

DNA extraction was carried out using the whole specimens with a DNeasy Blood and Tissue® Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The mtDNA *cox2* gene was amplified using the primers 210R: 5′-CAC CAA CTC TTA AAA TTA TC-3′ and 211F: 5′-TTT TCT AGT TAT ATA GAT TGR TTY AT-3′ (Nadler and Hudspeth, 2000). The PCR (polymerase chain reaction) reactions were set up in 50 µl reactions using 10 µl of DNA (≥ 10 ng) as a template, 1 µl (0.5 mM) of each primer, and 25 µl (2 ×) of HotStarTaq Master Mix (QIAGEN). The PCR was carried out using the following conditions: 95 °C for 15 min followed by 35 cycles at 94 °C for 30 s, 50 °C for 2 min and 72 °C for 2:30 min, followed by post amplification at 72 °C for

Table 2
Composition of samples used for comparative analyses on the distribution of *Anisakis* spp. and *Pseudoterranova cattani* in the South West Atlantic, including number of examined hosts (N); Latitude S (Lat) and Longitude W (Long) of capture; year and depth of capture; mean total length (MTL) of hosts; Prevalence (P); Mean abundance (MA), with confidence intervals (95%) between parentheses.

Host species	Sample code	N	Lat[a]	Long[a]	Year	Depth[b]	MTL[c]	*Anisakis* spp.		*Pseudoterranova cattani*	
								P	MA	P	MA
Z. chilensis	Zc1	15	36.80	54.72	2012	84.4	59.6	13.3 (1.7–40.5)	0.2 (0.0–0.5)	6.7 (0.2–31.9)	0.1 (0.0–0.4)
	Zc2	31	38.99	57.85	2011	72.5	68.0	45.2 (27.3–63.9)	0.8 (0.4–2.1)	16.1 (5.5–33.7)	1.4 (0.1–6.2)
	Zc3	31	45.85	61.74	2012	100.5	60.6	61.3 (42.2–78.2)	9.8 (4.0–26.3)	22.6 (9.6–41.1)	3.3 (0.1–12.8)
	Zc4	9	49.16	66.09	2012	108.1	65.0	33.3 (7.5–70.1)	1.9 (0.2–6.6)	22.2 (2.8–60.0)	0.6 (0.0–1.8)
S. bonapartii	Sb1	18	34.77	55.36	2013	22.4	64.9	0.0 (0.0–18.5)	0.0	0.0 (0.0–18.5)	0.0
	Sb2	33	35.84	53.64	2012	83.8	56.9	21.2 (8.9–39.9)	0.4 (0.1–1.3)	0.0 (0.0–10.6)	0.0
	Sb3	8	36.17	54.06	2012	77.5	57.3	37.5 (8.5–75.5)	0.4 (0.0–0.6)	0.0 (0.0–37.0)	0.0
	Sb4[d]	30	37.42	56.54	2010	30.0	64.5	10.0 (2.1–26.5)	0.1 (0.0–0.2)	0.0 (0.0–11.6)	0.0
	Sb5	9	38.43	57.47	2012	65.3	63.9	55.6 (21.2–86.3)	1.1 (0.3–2.9)	33.3 (7.5–70.1)	0.4 (0.0–0.9)
	Sb6[d]	44	38.53	59.22	2010	20.0	54.3	27.3 (14.9–42.8)	0.4 (0.2–0.7)	2.3 (0.1–12.0)	0.02 (0.0–0.1)
	Sb7[d]	11	39.01	61.5	2015	5.0	46.8	0.0 (0.0–28.5)	0.0	9.1 (0.2–41.3)	0.2 (0.0–0.6)
	Sb8	18	39.14	60.56	2011	15.6	47.4	0.0 (0.0–18.5)	0.0	5.6 (0.1–27.3)	1.4 (0.0–4.2)
	Sb9[d]	35	41.30	64.22	2015	50.0	62.3	85.7 (69.7–95.2)	17.9 (0.8–69.1)	45.7 (28.8–63.4)	1.1 (0.4–3.5)
	Sb10	12	46.49	66.23	2012	52.2	52.5	75.0 (42.8–94.5)	7.7 (0.6–28.6)	16.7 (2.1–48.4)	0.4 (0.0–1.1)
A. castelnaui	Ac1[d]	30	36.41	55.15	2017	48.0	85.2	3.3 (0.1–17.2)	0.03 (0.0–0.1)	40.0 (22.7–59.4)	2.5 (1.0–5.7)
	Ac2[d]	10	37.25	56.25	2017	60.0	76.6	0.0 (0.0–30.9)	0.0	10.0 (0.1–44.5)	0.1 (0.0–0.3)
	Ac3[d]	7	37.56	56.57	2016	40.0	89.6	0.0 (0.0–41.0)	0.0	100.0 (59.0–100.0)	11.4 (1.0–32.3)

[a] Central point of distribution when two or more trawls were made.
[b] Average value (m) for samples from research cruises.
[c] Average value (cm).
[d] Commercial trawlers.

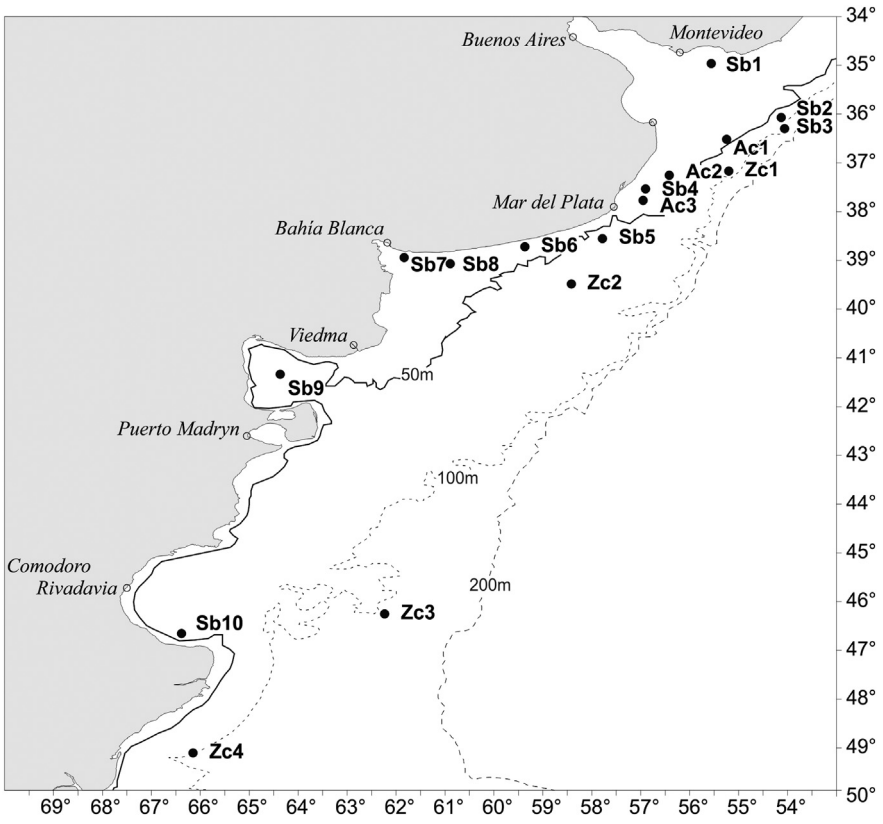

Fig. 1. Map showing the sampling localities in the Argentine Sea. Locality codes as indicated in Table 2.

10 min (Valentini et al., 2006). Each PCR product was purified using QIAquick spin columns (QIAquick Gel Extraction Kit, QIAGEN). The fragments were sequenced for both DNA strands using the PCR primers. Sequencing was performed using Big Dye Terminator vs. 3.1 and 3130xl Genetic analyzer (Applied Biosystem, Foster City, CA) at the Genomic Unit, IB-INTA.

2.4. Sequence analysis

Sequences were edited and assembled manually in Proseq 3.5 (Filatov, 2009). For the identification, the obtained sequences were analyzed by BLAST algorithm (Basic Local Alignment Search Tool), following default parameters (Altschul et al., 1990) and then aligned based on their inferred (in silico-translated) protein with available *cox2* mtDNA sequences for members of Anisakidae by ClustalW (Thompson et al., 1994) implemented in the MEGA 7.0 software package (Kumar et al., 2016), using default parameters. All sequences were deposited in the GenBank. Accession numbers are indicated in Table 3.

2.5. Distribution patterns of larval anisakids

Prevalence and mean abundance of *Anisakis* spp. and *Pseudoterranova* sp. were calculated following Bush et al. (1997) for each sample. Sterne's exact 95% confidence limits were calculated for prevalence and mean abundance using Quantitative Parasitology 3.0 software (QP3.0) (Rózsa et al., 2000; Reiczigel, 2003).

To analyze the relative contribution of host/abiotic variables on parasites distribution, Euclidean distance matrices of both prevalence and mean abundance were analysed by distance-based multiple linear regressions (DistLM) (Anderson et al., 2008) with significance testing based on 9999 permutations. Despite two species of *Anisakis* were genetically identified, quantitative analyses were carried out considering all specimens as belonging to the same taxon due to most worms were not identified at specific level and because *A. berlandi* seems to represent a minor proportion of the sample regarding *A. pegreffii* (see Results). The following host-related predictor variables were included in the models: the host species since the three species display different diets (Paesch, 2000) and their mean total length due to known influence of host size on parasite burdens (Braicovich et al., 2016; Timi and Lanfranchi, 2013; Timi et al., 2011). Abiotic predictor variables were latitude, longitude and depth of capture because they have been reported as determinants of anisakid burdens in bony fish in the region (Cantatore and Timi, 2015; Timi, 2003; Timi et al., 2014), year of capture was also included as predictor to account for possible temporal variation in parasite burdens since samples were caught between 2010 and 2017. Draftsman plots and correlation matrices were used to check for multicollinearity in the predictor variables; latitude and longitude were highly correlated each other ($R = 0.90$), due to the north-east to southwest orientation of the Argentine continental shelf, therefore only

Table 3
GenBank accession numbers for specimens of *Anisakis* and *Pseudoterranova* collected from different skates and localities (abbreviations correspond to Fig. 1).

Parasite species	Host	Locality	GenBank accession no.
Anisakis berlandi	*Sympterygia bonapartii*	Sb3	MF353876
Anisakis pegreffii	*Sympterygia bonapartii*	Sb3	MF353877–MF353880
	Sympterygia bonapartii	Sb5	MF353881, MF353882
	Sympterygia bonapartii	Sb 9	MF353883–MF353885
	Sympterygia bonapartii	Sb10	MF353886–MF353891
	Zearaja chilensis	Zc1	MF353892
	Zearaja chilensis	Zc2	MF353893, MF353894
	Zearaja chilensis	Zc3	MF353895–MF353897
Pseudoterranova cattani	*Atlantoraja castelnaui*	Ac1	MF353898
	Sympterygia bonapartii	Sb5	MF353899

latitude was included in the analyses. Models including all possible combinations of predictor variables were generated using the Best procedure within the DistLM routine. An information theoretic approach based on modified Akaike's Information Criterion (AICc) was used to identify the best model; models with the lowest AICc were considered the most parsimonious (Symonds and Moussalli, 2011). Models with Δ_i between 0 and 2 are considered as having a substantial level of empirical support of the model being therefore as good as the best model (Burnham and Anderson, 2002), however as suggested by Richards (2005) models with $\Delta_i \leq 6$ were retained. For each of selected models, the Akaike weights (w_i) were calculated following Burnham and Anderson (2002) to identify and quantify the uncertainty in model selection and further used to estimate the relative importance of each predictor variable (predictor weight). For each predictor, the Akaike weights of all the models (with $\Delta_i < 6$) that contained that predictor were summed and that values were interpreted as the relative importance of that predictor (Symonds and Moussalli, 2011). Also the relative strengths of each candidate model was assessed by calculating the evidence ratio (ER), which provides a measure of how much more likely the best model is than alternative models (Burnham and Anderson, 2002). Multivariate analyses were implemented in PERMANOVA + for PRIMER7 package (Anderson et al., 2008; Clarke and Gorley, 2015).

3. Results

3.1. General results

Third stage larvae of *Anisakis* and *Pseudoterranova* were found in the three host species (Table 2, Fig. 2).

In all parasitized hosts, but one, parasites were found in the stomach wall, the exception being a specimen of *S. bonapartii* harbouring a single larval *Anisakis* in the liver parenchyma. No parasites were found in musculature. Based on morphologic and morphometric data (not shown), all of larval *Pseudoterranova* were identified as *P. cattani*, the unique species so far known in the study region.

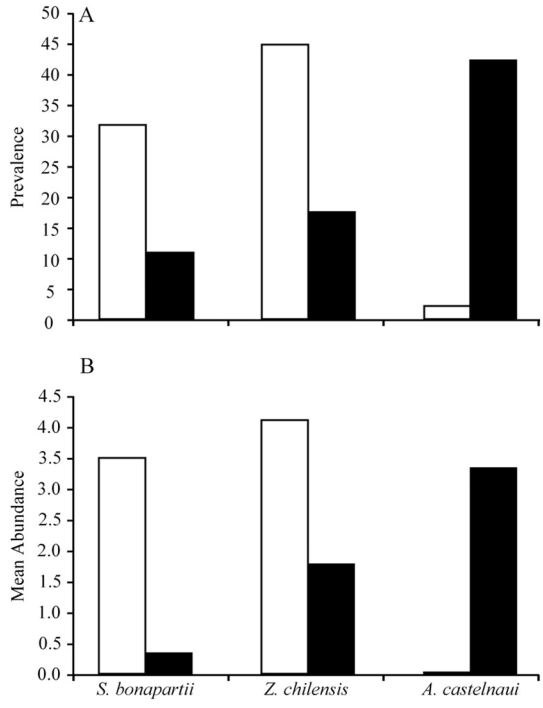

Fig. 2. Prevalence (A) and mean abundance (B) of *Anisakis* spp. (white bars) and *Pseudoterranova cattani* (black bars) in three skates species from the Argentine Sea.

3.2. Nematode species identification and sequence analysis

The mtDNA *cox2* sequences were determined for a total of 24 larval anisakids isolated from the three rajid species. The length of the trimmed sequences were 582 bp for *Anisakis* fragment and 504 bp for *Pseudoterranova* one. The identification through BLAST and ClustalW showed that 21 specimens belonged to *A. pegreffii* and one to *A. berlandi*. In agreement with morphological results, BLAST results of the two *Pseudoterranova* larvae revealed that specimens belong to *P. cattani* (Table 3).

3.3. Distribution of larval anisakids

Prevalence and abundance of larval anisakids varied across samples (Table 2, Fig. 2). In the case of *Anisakis*, the results of the DistLM on the prevalence data showed that the best model included only latitude as predictor variable (explaining 34% of the total variation of data) (Table 4). The w_i indicated that it has 31% chance of being the best model and ER showed that it was near two and a half times more likely to be the best approximating model than the subsequent one. Indeed, latitude was included in most models with $\Delta_i < 6$ reaching a predictor weight of 0.84, which indicates that this variable had the highest probability of being a component of the best model (Fig. 3A). Regarding mean abundance, eleven alternative models were obtained, the best one also composed only by latitude as predictor variable (explaining near 27% of the total variation of data) (Table 4). The w_i indicated that the first model has a 44% chance of being the best one, a value more than twice higher to that of the subsequent model (composed by latitude and year). The predictor weights indicated that latitude, with a value of 0.91, had the highest relative importance as predictor of mean abundance (Fig. 3B).

Regarding *P. cattani*, the results of the DistLM on the prevalence data showed that the best model included only mean host size as predictor variable (explaining 46% of the total variation of data) (Table 4). The w_i indicated that it has 31% chance of being the best model and ER showed that it was near one and a half times more likely to be the best approximating model than the subsequent one. Indeed, host size was included in all, but one, models with $\Delta_i < 6$ reaching a predictor weight of 0.97, which indicates that this variable had the highest probability of being a component of the best model (Fig. 3A). When mean abundance was analysed, the best model was also composed only by mean host size as predictor variable (explaining 39% of the total variation of data) (Table 4). The w_i indicated that the first model has a 45% chance of being the best one, a value three times higher to that of the subsequent model (composed by mean host size and latitude). The predictor weights indicated that host size, with a value of 0.91, had the highest relative importance as predictor of mean abundance (Fig. 3B).

4. Discussion

It has been postulated that the presence of larval nematodes in the elasmobranchs body cavity and tissues is unusual, due to the high concentrations of urea (Caira and Healy, 2004; Moya et al., 2015). However, according to the literature, it appears to be that larval *Anisakis* and *Pseudoterranova* are common components of parasite assemblages for this group of hosts. Indeed, elasmobranchs may not represent a dead-end for the life cycle of these parasites, since shark and rays have been reported as usual preys of cetacean definitive hosts (Visser et al., 2000).

This is the first genetically confirmed record of *A. pegreffii*, *A. berlandi* and *P. cattani* parasitizing batoid hosts; and, for the last two species, the first record in elasmobranchs. Most of previous records of larval anisakids in chondrichthyans are based on morphological identifications, with only *A. simplex s.s.* and *A. physeteris* being diagnosed by molecular tools in the gastrointestinal serosa and body cavity of several shark species from Azores Islands (Kuhn et al., 2011; T. Kuhn pers.

Table 4
Summary table of the results of the DISTLM analysis on prevalence and mean abundance of *Anisakis* spp. and *Pseudoterranova cattani* in 17 samples corresponding to 3 skate species from the South West Atlantic. Results are ordered by the modified Akaike information criterion and only those models with $\Delta_i < 6$ included.

Response variable	*Anisakis* spp.							*Pseudoterranova cattani*						
	Model	AICc	R^2	Predictors	Δ_i	w_i	ER	Model	AICc	R^2	Predictors	Δ_i	w_i	ER
Prevalence	PA1	110.61	0.34	3	0	0.3135	–	PP1	103.09	0.46	1	0	0.3094	–
	PA2	112.49	0.38	2, 3	1.88	0.1225	2.56	PP2	103.62	0.53	1, 3	0.53	0.2374	1.30
	PA3	113.00	0.36	3, 4	2.39	0.0949	3.30	PP3	105.03	0.59	1, 2, 3	1.94	0.1173	2.64
	PA4	113.21	0.35	1, 3	2.60	0.0854	3.67	PP4	105.19	0.49	1, 4	2.10	0.1083	2.86
	PA5	113.87	0.57	2, 3, 5	3.26	0.0614	5.10	PP5	105.85	0.57	1, 3, 4	2.76	0.0778	3.97
	PA6	114.24	0.44	3, 5	3.63	0.0511	6.14	PP6	105.86	0.47	1, 2	2.77	0.0774	3.99
	PA7	114.25	0.18	2	3.64	0.0508	6.17	PP7	107.62	0.30	4	4.53	0.0321	9.63
	PA8	115.01	0.41	1, 2, 3	4.40	0.0347	9.03	PP8	108.49	0.60	1, 2, 3, 4	5.40	0.0208	14.88
	PA9	115.07	0.28	1, 2	4.46	0.0337	9.30	PP9	108.62	0.49	1, 2, 4	5.53	0.0195	15.88
	PA10	115.32	0.53	1, 3, 5	4.71	0.0298	10.54							
	PA11	115.41	0.40	2, 3, 4	4.80	0.0284	11.02							
	PA12	115.54	0.40	2, 5	4.93	0.0267	11.76							
	PA13	116.34	0.22	2, 4	5.73	0.0179	17.55							
	PA14	116.44	0.36	1, 3, 4	5.83	0.0170	18.45							
	PA15	116.52	0.06	4	5.91	0.0163	19.20							
	PA16	116.58	0.06	1	5.97	0.0158	19.79							
Mean abundance	MA1	52.42	0.27	3	0	0.4394	–	MP1	30.06	0.39	1	0	0.4464	–
	MA2	54.57	0.31	3, 4	2.15	0.1499	2.93	MP2	32.41	0.41	1, 3	2.35	0.1379	3.24
	MA3	55.39	0.27	1, 3	2.97	0.0994	4.42	MP3	32.55	0.41	1, 2	2.50	0.1280	3.49
	MA4	55.40	0.27	2, 3	2.98	0.0989	4.44	MP4	33.03	0.39	1, 4	2.98	0.1007	0.43
	MA5	57.16	0.04	2	4.74	0.0411	10.68	MP5	34.07	0.35	5	4.01	0.0600	7.44
	MA6	57.31	0.48	3, 4, 5	4.89	0.0381	11.54	MP6	34.35	0.46	1, 2, 3	4.30	0.0520	8.58
	MA7	57.34	0.34	1, 3, 4	4.92	0.0376	11.69	MP7	35.59	0.16	4	5.53	0.0281	15.90
	MA8	57.51	0.02	1	5.09	0.0345	12.73	MP8	35.87	0.41	1, 3, 4	5.82	0.0244	18.31
	MA9	57.69	0.01	4	5.27	0.0315	13.95	MP9	36.03	0.41	1, 2, 4	5.98	0.0225	19.86
	MA10	57.80	0.32	3, 5	5.39	0.0297	14.78							
	MA11	58.05	0.31	2, 3, 4	5.64	0.0262	16.74							

AICc modified Akaike information criterion; R^2 proportion of explained variation for the model; Predictor variables: 1 - mean host length; 2 - depth; 3 - latitude; 4 - year; 5 - host species. Δ_i difference between the AICc of the best model and the AICc for each of the other models; w_i Akaike weight; ER evidence ratio.

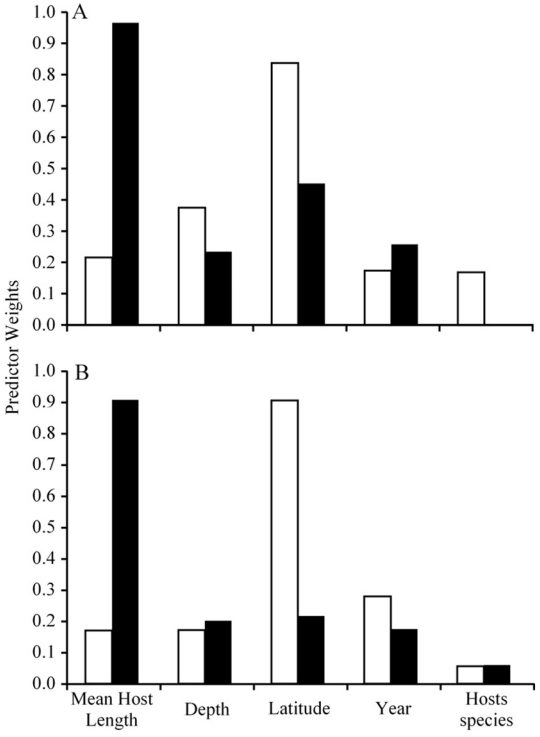

Fig. 3. Predictor weights of variables included in models with $\Delta_i < 6$ resulting of the DISTLM analyses on prevalence (A) and Mean Abundance (B) of *Anisakis* spp. (white bars) and *Pseudoterranova cattani* (black bars) in 17 samples corresponding to 3 skate species from the Argentine Sea.

com.). On the other hand, *A. simplex s.s.*, *A. pegreffii* and *Pseudoterranova ceticola* were identified in the gastrointestinal lumen of some shark species from Madeira Archipelago and the Adriatic Sea (Costa et al., 2014; Gračan et al., 2016), which probably are accidental and transient parasites in these hosts.

Whereas *A. pegreffii* and *P. cattani* have been previously reported in teleost hosts in the study region (Mattiucci and Nascetti, 2008; Timi et al., 2014), *A. berlandi*, previously known as *A. simplex* C (Mattiucci et al., 1997, 2014), is only known as larvae and adults from the North and South Pacific (Canada, Chile, Australia and New Zealand), the South Shetland Islands and the South African Atlantic coast (Kuhn et al., 2011; Mattiucci et al., 2014). However, the record of *A. berlandi* in southwestern Atlantic waters widens its distribution range, including a region where its main definitive host, *Globicephala melas*, is also distributed (Rice, 1998).

Taking into account recent reports from Korea (Sohn et al., 2015) that refer to skates as probable sources of anisakid infestation in humans and considering that both *A. pegreffii* and *P. cattani* are recognized as causative agents of human anisakidosis (Lim et al., 2015; Mattiucci et al., 2013, 2017b; Timi et al., 2014; Torres et al., 2007), they could potentially be a zoonotic hazard to skate meat consumers. On the other hand, no data concerning the possible infectiveness in humans of *A. berlandi* are available (Mattiucci et al., 2017b). In teleost fishes, anisakid third-stage larvae usually parasitize the body cavity, viscera and musculature (Buchmann and Mehrdana, 2016); being their presence in fillets the most common source of infestation for humans. However, in the present study, larvae of both genera were restricted to the stomach wall of skates, with only one host harbouring a single larva in the liver, but no infestations in skeletal musculature, the edible part of the fish, were observed. These findings agree with most reports of larval anisakids in elasmobranchs, in which the stomach wall is the most reported microhabitat for these parasites (see Table 1). This represents a low risk of contracting anisakidosis by consumption of elasmobranch meat.

Furthermore, unlike bony fishes, in which larvae migration to the fillet is commonly reported after host death (Cipriani et al., 2016), no migrating or free larvae were observed in skates, although several of them were preserved in ice or refrigerator for many hours to few days after capture. Even if experimental work to assess the occurrence of post-mortem larval migration in elasmobranchs has not been carried out yet, the risk of consuming meat containing infective larvae seems to be low in the case of skate species here studied.

Host features are generally recognized as composition and structure determinants of parasite populations and communities. In that sense, the observed variability of parasite prevalence and mean abundance across the three species could be related to their different feeding habits, bathymetric distribution and body size. *Sympterygia bonapartii* is mainly carcinophagous (Estalles et al., 2016; Paesch, 2000), whereas fishes largely dominate the *A. castelnaui* diet (Barbini and Lucifora, 2012; Paesch, 2000). Finally, *Z. chilensis* diet is composed of both kinds of preys (Paesch, 2000; Belleggia et al., 2016). Apparently, increasing ichthyophagy across host species could be related to higher burdens of *P. cattani*, but not to population descriptors of *Anisakis* spp. Other variables differing among host species could also account for the observed patterns. Indeed, whereas *Z. chilensis* lives in deep waters, *A. castelnaui* and *S. bonapartii* are mostly coastal species (Cousseau and Perrotta, 2013). However, the latter species displays a migratory behaviour, alternating between coastal estuarine areas and the coastal area down to 50 m (Mabragaña et al., 2002). Other characteristics, such as differences in fish size, can also play a role in the observed patterns, especially considering that larval anisakids are long-lived and tend to exhibit cumulative patterns as fish grow (Braicovich et al., 2016; Timi et al., 2011), e.g., *A. castelnaui* is significantly larger than the other two species.

Beyond host features, parasites geographical distribution is also a relevant driver of parasite burdens in fish. Environmental conditions can influence parasite distribution, either directly or indirectly, through their effects on the distribution of hosts. For marine parasites in particular, geographical distributions are mainly determined by temperature-salinity profiles and their association to specific masses of water (Esch and Fernández, 1993). Anisakids show species-specific distributions within different climate zones and oceans (Kuhn et al., 2011). In the Southern Hemisphere, both *A. pegreffii* and *A. berlandi* inhabit cold waters of the southern regions (Klimpel and Palm, 2011; Mattiucci and Nascetti, 2008; Mattiucci et al., 2017b). In accordance with global patterns, prevalence and mean abundance of larval *Anisakis* in teleost fishes from the Argentine Sea follow a latitudinal pattern increasing southwards, irrespective of the host species harbouring them (Cantatore and Timi, 2015).

On the other hand, *P. cattani* is distributed along southern Pacific and Atlantic coasts in South America, following the distribution of its definitive host, the sea lion *Otaria flavescens* (Timi et al., 2014). In Atlantic waters fishes, this species also shows increasing burdens southwards; where larvae are more common and have been reported in hosts free of these parasites in northern waters (Timi et al., 2014).

These latitudinal patterns are congruent with the environmental conditions of the study region, mostly with the temperature cline (decreasing southwards) characteristic of the area (Hoffmann et al., 1997; Piola et al., 2010). Temperature also decreases with depth at lower latitudes (Acha et al., 2004; Piola et al., 2010).

Undoubtedly, a combination of several interacting variables determines the distribution of anisakids in skates, whose relative effect was proven by multivariate analyses. Despite the fact that host species seemed to play a role on parasite loads when population parameters were averaged for each species, this variable was of little relevance regarding other predictors when all fish samples were analysed together. The limited influence of host species on parasite burdens indicates that skates act as passive samplers of infective stages available in their habitat. Parasite prevalence and abundance are modelled, therefore, by the trophic level and dietary preferences of skates, as well as by the environmental conditions determining parasite distribution.

In the case of *Anisakis* spp., both prevalence and mean abundance were largely determined by latitude, which as a surrogate of water temperature, demonstrates that the effect of environmental conditions prevails as a determinant of parasite distribution. The low values of these parasites in *A. castelnaui* samples could be a consequence of the comparatively lower number of examined fish, but could also be related to the fact that only skates from the northern region coastal waters were examined. On the other hand, *S. bonapartii* is also a mainly coastal species, but its seasonal migrations to deeper and cooler waters explain the higher levels of parasitism by *Anisakis* larvae. Finally, *Z. chilensis* lives in deep waters along its distribution range and shows the highest values of prevalence and abundance for larval *Anisakis*. As in the case of latitude, depth can be considered as a surrogate of water temperature, especially in the northern region of the study area.

Regarding *P. cattani*, the main driver of prevalence and abundance was host size. The transmission of this species in the region is favoured by a combination of intermediate/paratenic host traits that includes large size, high trophic level and benthophagic habits (Timi et al., 2014). Due to the ichthyophagous habits of *A. castelnaui* and its large size, this skate is prone to consume infested preys that are not included in the diet of the other two species.

In conclusion, larvae of three the species of anisakids, two of them having a pathogenic potential for humans, were found parasitizing skates in the southwestern Atlantic. The results of this study proved differential distribution patterns between anisakid genera in skates from the study region, with levels of parasitism by *Anisakis* spp. increasing towards southern and deeper waters. On the other hand, those of *P. cattani* increased with the host size. The parasites microhabitat, the stomach wall, and the lack of evidence for post-mortem migrations suggest that there is a low risk of infestation for the consumer health.

However, considering that the intake of these parasites, even dead worms or their allergens, can result in allergic reactions (Ivanović et al., 2017), the mere presence of worms or their antigens in fish meat can represent a health hazard.

Based on this information, measures to decrease the possibilities of contact with parasites or their allergens can be taken during harvesting including avoidance of southern fishing regions, large-sized skates, or even particular skate species.

Acknowledgements

Thanks are extended to the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) and the Industrias El Corsario A.A., Mar del Plata for providing fish samples; to Dr. Santiago Barbini and Lic. David Sabadin (IIMyC-UNMdP) for helping during samples processing, the identification of skates and providing literature on chondrichthyans. Financial support was provided by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP No. 112-201501-00973), the Fondo para la Investigación Científica y Tecnológica (PICT 2015 No. 2013) and the Universidad Nacional de Mar del Plata (EXA 869/16). This work is part of the Doctoral Thesis of M.M.I., carried out in the Universidad Nacional de Mar del Plata, Facultad de Ciencias Exactas y Naturales.

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