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Molecular identification, morphological characterization and new insights into the ecology of larval *Pseudoterranova cattani* in fishes from the Argentine coast with its differentiation from the Antarctic species, *P. decipiens* sp. E (Nematoda: Anisakidae)

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ABSTRACT

Larvae of the genus *Pseudoterranova* constitute a risk for human health when ingested through raw or undercooked fish. They can provoke pseudoterranovosis in humans, a fish-borne zoonotic disease whose pathogenicity varies with the species involved, making their correct specific identification a necessary step in the knowledge of this zoonosis. Larvae of *Pseudoterranova decipiens s.l.* have been reported in several fish species from off the Argentine coasts; however, there are no studies dealing with their specific identification in this region. Here, a genetic identification and morphological characterization of larval *Pseudoterranova* spp. from three fish species sampled from Argentine waters and from *Notothenia coriiceps* from Antarctic waters was carried out. Larvae were sequenced for their genetic/molecular identification, including the mitochondrial cytochrome *c* oxidase subunit II (mtDNA *cox2*), the first (ITS-1) and the second (ITS-2) internal transcribed spacers of the nuclear ribosomal DNA, and compared with all species of the *P. decipiens* (*sensu lato*) species complex (sequences available in GenBank). Further, adults of *Pseudoterranova* spp. from the definitive host, the southern sea lion, *Otaria flavescens*, from Argentine and Chilean coasts were sequenced at the same genes. The sequences obtained at the ITS-1 and ITS-2 genes from all the larvae examined from fish of Argentine waters, as well as the adult worms, matched 100% the sequences for the species *P. cattani*. The sequences obtained at mtDNA *cox2* gene for Antarctic larvae matched 99% those available in GenBank for the sibling

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P. decipiens sp. E. Both MP and BI phylogenetic trees strongly supported *P. cattani* and *P. decipiens* sp. E as two distinct phylogenetic lineages and depicted the species *P. decipiens* sp. E as sister taxon to the remaining taxa of the *P. decipiens* complex. Larval morphometry was similar between specimens of *P. cattani* from Argentina, but significantly different from those of *P. decipiens* sp. E, indicating that larval forms can be distinguished based on their morphology. *Pseudoterranova cattani* is common and abundant in a variety of fish species from Chile, whereas few host species harbour these larvae in Argentina where they show low levels of parasitism. This pattern could arise from a combination of factors, including environmental conditions, density and dietary preferences of definitive hosts and life-cycle pathways of the parasite. Finally, this study revealed that the life-cycle of *P. cattani* involves mainly demersal and benthic organisms, with a marked preference by large-sized benthophagous fish.

1. Introduction

Nematodes of the family Anisakidae have a worldwide distribution, occurring in fish species from all oceans and seas. Several anisakid genera are found as adults in marine mammals, and as larvae in diverse tissues of fish, including muscles, and in some invertebrates (Anderson, 1992; Mattiucci and Nascetti, 2008). Anisakid larvae are a potential risk to humans as they may cause a disease known as anisakidosis, whose main etiologic agents belong to the genera *Anisakis* Dujardin, 1845, and *Pseudoterranova* Mozgovoi, 1951 (see Mattiucci et al., 2013a,b for a review). Human infection occurs when raw, undercooked or marinated fish, containing living larvae is eaten; therefore, the study of anisakids is relevant for medicine, veterinary, food inspection and hygiene, legislation and fishery industry.

Larvae of the *Pseudoterranova* decipiens (Krabbe, 1878) species complex, known as "sealworms" or "codworms", are the second most common pathogen among anisakids reported from humans after the species of the *Anisakis simplex* complex. Nematodes of the genus *Pseudoterranova* have proven to be not only a costly cosmetic problem for seafood processors, but a risk for human health due the severe pathology they can cause when consumed with raw or undercooked fish (McClelland, 2002; Zhu et al., 2002; Mattiucci and Nascetti, 2008) provoking in humans the fish-borne zoonotic disease named Pseudoterranovosis.

The first reports of human infections by *P. decipiens* (*sensu lato.*) were from North America (Margolis, 1977; Lee et al., 1985), followed by cases described as transient infections in California. In Korea, human pseudoterranovosis was first described by Lee et al. (1985) and more recently several cases have been recorded (Mattiucci et al., 2013a for a review). Human cases of this zoonosis have also been registered in Chile, Japan, and Northern Europe. Recently, a human case due to the species *P. azarasi*, was reported in Japan (Arizono et al., 2011).

Adults of *P. decipiens* (sensu lato) are worldwide distributed parasites of phocid and otariid seals; this morphospecies comprises six biological species, genetically detected for the first time by allozymes (Paggi et al., 1991). These are *P. decipiens* (Krabbe, 1878) *s.s.*, *P. bulbosa* (Cobb, 1888), *P. azarasi* (Yamaguti et Arima, 1942), and *P. krabbei* Paggi, Mattiucci, Gibson, Berland, Nascetti, Cianchi et Bullini, 2000 from the Boreal and Arctic-Boreal regions, and two species from the southern hemisphere, namely *P. decipiens* sp. E from the Antarctica (Bullini et al., 1997) and *P. cattani* (George-Nascimento and Urrutia, 2000), a parasite of *Otaria flavescens* (=*O. byronia*) from Chile previously described as *Phocanema decipiens* (Cattan and Carvajal, 1980; Mattiucci et al., 1998; George-Nascimento and Urrutia, 2000; Paggi et al., 2000). Recently, other genetic/molecular markers have been used to distinguish the biological species of *Pseudoterranova* based on SSCP analysis of the ITS region of the rDNA (Zhu et al., 2002), while some of them have been also sequenced at the ITS region of the rDNA (Nadler et al., 2005). Moreover, *P. decipiens s.s.*, *P. krabbei*, *P. bulbosa*, *P. azarasi* and *P. decipiens* sp. E have been recently sequenced at the mtDNA *cox2* gene and sequences are deposited in GenBank (Mattiucci et al., 2013a). Other two species are recognized within the genus *Pseudoterranova*: *P. kogiae* (Johnston et Mawson, 1939) from the pygmy sperm whale, *Kogia breviceps* and *P. ceticola* (Deardorff and Overstreet, 1981) from the dwarf sperm whale, *K. sima* (Gibson, 1983); the latter has been sequenced at the mitochondrial mtDNA *cox2* (Valentini et al., 2006) and at the ITS region of the rDNA (Cavallero et al., 2011).

Third-stage larvae of *P. decipiens* are well known in several species of fish and invertebrates from different regions of the world (Margolis, 1977; McClelland et al., 1990; Bristow and Berland, 1992; Marcogliese, 1996; McClelland, 2002). Larval stages of this genus have been genetically identified to the species level in some fish species of the NE and NW Atlantic Ocean, as well as in gadids and flatfish collected in the North Pacific Ocean and Japanese Sea waters (Mattiucci et al., 1998). In South Western Atlantic waters, larvae belonging to this genus have been reported in several fish species, some of them being of high economic relevance. Nevertheless, and in spite of the number of previous records, there are no studies dealing with their specific identification based on genetic and morphological characterization.

The aim of this work was three-fold: (i) to identify, for the first time, based on both morphologic characters and genetic/molecular markers, the specific status of third-stage larvae of *Pseudoterranova* spp. found in fish from South-Western Atlantic waters, as this region still constitutes a gap in the known zoogeography of the *P. decipiens* species complex; (ii) to provide morphological characters for their recognition; (iii) to provide information on its ecology in Argentine waters.

2. Materials and methods

2.1. Samples collection and processing

Larval nematodes were obtained from three fish species caught by commercial trawlers in waters off Buenos Aires Province, Argentina, and landed at the ports of Mar del Plata and Quequén. Host species examined were: Argentine sea bass, *Acanthistius patachonicus* (Jenyns, 1842) (Serranidae): 45 specimens (39°–40°30′S/59°–60°30′ W; April, 2001; total length 35.6 ± 6.4 cm) and Argentine sandperch, *Pseudopercis semifasciata* (Cuvier, 1829) (Pinguipedidae): 30 specimens (38°03′–38°44′ S/57°30′–58°44′ W; November, 2007; total length 71.2 ± 3.5 cm), both species landed at Mar del Plata port; flounder, *Paralichthys patagonicus* Jordan, 1889 (Paralichthyidae): 51 specimens (38°52′S/58°10′ W; September, 2010; total length 35.20 ± 2.64) landed at Quequén Port. Fish were kept fresh or deeply frozen in plastic bags at −18 °C until analysis. Each fish was measured for total length, sexed and examined for anisakid nematodes. Viscera (stomach, intestine, liver, coelomatic cavity and mesenteries) were examined under stereoscopic microscopy. Fish were filleted and fillets were sliced in thin sections and observed by candling, evidencing easily the large and brownish worms. Nematodes were collected and quantified, recording their microhabitat. Prevalence and mean intensity *sensu* Bush et al. (1997) were calculated following Rósza et al. (2000). After quantification, a subsample of larvae from each host species was used for morphometric measurements and another subsample for genetic analyses (see detail in Table 1). Additionally, a sample of 12 nematodes of the genus *Pseudoterranova* recovered from the body cavity and liver of *Notothenia coriiceps* Richardson, 1844 (Nototheniidae) from South Shetland Islands (Antarctic), available in our personal collections, was also examined for morphological comparative purposes (10 specimens), while the remaining two specimens were sequenced for genetic analyses.

Three male specimens of southern sea lion, *O. flavescens*, found dead on beaches of the Buenos Aires province, were examined for parasites, one from Mar del Plata (217 cm long, May, 2009) and two from Quequén (235 cm long, March 2011; 232 cm long, May 2011). Whereas all the three sea lions were found parasitized by hundreds of specimens of *Contracaecum* spp., only one harboured 11 adult specimens of *Pseudoterranova* sp. Eight nematodes of *Pseudoterranova* spp. from the last sea lion, together with a subsample of larvae from each fish species, were washed in physiological saline and fixed in 96% ethanol for molecular analyses (Table 1). Finally, six adult specimens of *Pseudoterranova* spp. from *O. flavescens* (five from Chioé, Central Chilean coast and one from Patagonian coast), available in our personal collections, were sequenced at nuclear and mitochondrial genes for their identification to the species level and genetic comparative analysis (Table 1).

The following sequences were obtained from the nematodes analyzed: the mitochondrial cytochrome *c* oxidase subunit II (mtDNA *cox2*) gene; the internal transcribed spacer 1 of ITS region of the nuclear ribosomal DNA (ITS-1

Table 1
Number of specimens of *Pseudoterranova cattani* and *P. decipiens* E genetically identified based on ITS rDNA and mtDNA *cox2*. Their hosts, collection locality, life-history stage and specimen' codes reported throughout the paper (see Section 2).

Host	Collection locality	Life-history stage	*N* ITS and mtDNA*cox2* with specimen code	GenBank accession number of mtDNA *cox2*	GenBank accession number of ITS of rDNA[a]
Otaria flavescens	Concepción (Chile)	Adult	5 (PCA1–PCA5)	PCA5 = KC782949	PCA5 = KC970079
Otaria flavescens	Patagonia (Argentina)	Adult	1 (PCA8)	PCA8 = JX500060	PCA8 = KC970078
Otaria flavescens	Quequén (Argentina)	Adult	8 (PCA16–PCA23)	PCA22 = JX500061	–
Paralichthys patagonicus	Quequén (Argentina)	L3	9 (PCA9–PCA12; PCA28–PCA32)	PCA9 = JX500058	PCA9 = KC970080
Pseudopercis semifasciata	Mar del Plata (Argentina)	L3	3 (PCA13–PCA15)	PCA13 = JX500059	PCA13 = KC970081
Acanthistius patachonicus	Mar del Plata (Argentina)	L3	6 (PCA6–PCA7; PCA24–PCA27)	PCA 6 = KC782947	PCA6 = KC970082
Notothenia coriiceps	South Shetlands (Antarctica)	L3	2 (PDE2–PDE3)	PDE2 = KC782948	PDE = KF017610

[a] The deposited sequences include the entire region ITS (ITS-1, 5.8S, ITS-2) of the rDNA. Only one sequence for each sample was deposited under the accession number, because the remaining were identical at the intraspecific level.

rDNA); the internal transcribed spacer 2 of the ITS region of nuclear ribosomal DNA (ITS-2 rDNA).

The total DNA was extracted from 2 mg of tissue from each single nematode by using cetyltrithylammonium bromide method (CTAB) as previously described in detail by Valentini et al. (2006) and Mattiucci et al. (2013a).

The mitochondrial cytochrome *c* oxidase subunit II (*cox2*) gene was amplified using the primers 211F (5′-TTT TCT AGT TAT ATA GAT TGR TTY AT-3′) and 210R (5′-CAC CAA CTC TTA AAA TTA TC-3′) (Nadler and Hudspeth, 2000) spanning the mtDNA nucleotide position 10,639–11,248, as defined in *Ascaris suum* [GenBank X54253]. The PCR (polymerase chain reaction) was carried out using the following conditions: 94 °C for 3 min (initial denaturation), followed by 34 cycles at 94 °C for 30 s (denaturation), 46 °C for 60 s (annealing), 72 °C for 90 s (extension), followed by post amplification at 72 °C for 10 min.

For the ITS region of the rDNA, PCR (polymerase chain reaction) amplification was performed using the primers NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′), according to the procedure reported in Zhu et al. (2002). PCR amplification conditions were: 94 °C for 5 min (initial denaturation), followed by 30 cycles at 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 30 s (extension) and a final elongation step at 72 °C for 5 min (Zhu et al., 2002).

2.2. Sequences analysis

The sequences obtained at the ITS-1 and ITS-2 of rDNA and mtDNA *cox2* for all the larval and adult specimens of *Pseudoterranova* spp. analyzed in the present study, were compared with those already obtained for the same genes in the species of the *P. decipiens* species complex appeared in previous studies (Zhu et al., 2002; Nadler et al., 2005). Therefore, the following sequences at the ITS-1 and ITS-2 of the species of the genus *Pseudoterranova*, retrievable from GenBank, were used for the identification of all the specimens examined: *P. cattani* (ITS-1: AJ413981; ITS-2: AJ413983), *P. decipiens s. s.* (ITS-1: AJ413968; ITS-2: AJ413967), *P. krabbei* (ITS-1: AJ413965; ITS-2: AJ413966), *P. bulbosa* (ITS1: AJ413969; ITS-2: AJ413972), *P. azarasi* (ITS-1: AJ413973; ITS-2: AJ413974). Similarly, the following mtDNA *cox2* sequences of the species of the genus *Pseudoterranova*, retrievable from GenBank, were used for the identification of all the specimens examined in the present study: *P. decipiens s.s.* (HM147278), *P. krabbei* (HM147279), *P. bulbosa* (HM147280), *P. azarasi* (HM147281), *P. decipiens* sp. E (HM147282).

The phylogenetic analysis on the sequences data sets obtained at all the *Pseudoterranova* spp. specimens examined was carried out by Maximum Parsimony (MP) and Bayesian analysis (BI) by using PAUP* (Swofford, 2003) and MrBayes3.1 (Huelsenbeck and Ronquist, 2005), respectively. Maximum Parsimony (MP) analysis was performed by using the heuristic search with tree-bisection-reconnection (TBR) branch-swapping algorithm; the reliabilities of the phylogenetic relationships were evaluated using nonparametric bootstrap analysis on 1000 pseudoreplicates (Felsenstein, 1985). Bootstrap values ≥70 were considered well supported (Hillis and Bull, 1993).

Bayesian Inference (BI), was performed by using the best fit substitution model implemented in jModetest3.1 (Huelsenbeck and Ronquist, 2005) over 88 possible substitution models, based on the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). The Bayesian analysis (BI) used the MCMC algorithm: the number of chains was 4, the temperature of heated chains was 0.2, the number of generations was 1,000,000 and the sub-sampling frequency was 100, with a burn-in fraction of 0.25. Posterior probabilities (*Pp*) were estimated after discarding trees as "burn-in" and used to assess support for each branch in the phylogeny; clades with *Pp* ≥95% (expressed as percentages) were considered strongly (significantly) supported (Reeder, 2003). *Anisakis pegreffii* and *Ascaris suum* were used as outgroups.

2.3. Comparative morphometric analysis

Subsamples of parasites of *A. patachonicus* (30 worms), *P. patagonicus* (10 worms), *P. semifasciata* (10 worms) and *N. coriiceps* (10 worms) were fixed in ethanol 70% for storage. For light microscopy analysis, nematodes were cleared in lactophenol (Berland, 1984).

The nematodes were measured, data of each worm were related to its total length and the differences observed among worms from different host species were examined using canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003; Anderson et al., 2008), based on Euclidean distances. To test for significant differences between the samples, a permutation "trace" test (sum of squared canonical Eigen values) was applied, and *P* was obtained after 9999 permutations. An indication of the underlying morphometric features drawing differences between groups was obtained by the strength of their correlation with the canonical discriminant axes coordinates, with diagnostic compounds visualized using vector overlays based on Pearson correlations.

Differences in morphometric relationships of nematodes across fish species were examined in more detail by mean of a one-way PERMANOVA, with host species as fixed factor after 9999 permutations based on Euclidean distances. When differences were detected by PERMANOVA, pair-wise comparisons were used to determine which groups differed. Following Anderson et al. (2008) a permutation of residuals under an unrestricted model was used as method of permutation. A sequential sum of squares (Type I SS) was applied because samples were unbalanced (different numbers of nematodes measured by sample). All multivariate statistical procedures on parasite community data were performed by PRIMER V6 (Clarke and Gorley, 2006; Clarke and Warwick, 2001) and PERMANOVA+ for PRIMER (Anderson et al., 2008) packages.

2.4. Distribution of larvae across fish species in the region

The distribution of larvae across fish species with different habits, size, and trophic level inhabiting waters off the northern Argentine Sea (Buenos Aires province) was analyzed based on bibliographic data as well as on unpublished data from the authors. The trophic level values of each fish species were obtained from FishBase, which provides TL estimates from food items (Froese and Pauly, 2013).

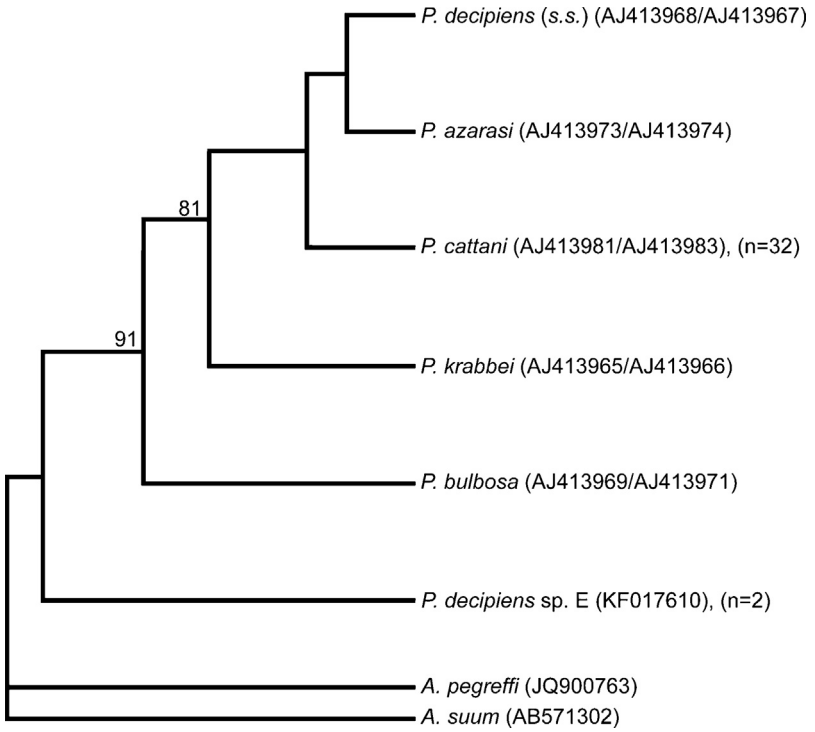

Fig. 1. Maximum parsimony (MP) bootstrap consensus tree by PAUP*4.0 (bootstrap method with heuristic search) (Swofford, 2003) inferred from the sequences of ITS-1 and ITS-2 rDNA of *P. cattani* and *P. decipiens* sp. E analyzed in the present study, with respect to the other species of the *P. decipiens* (*s.l.*) complex, sequenced for the same gene and deposited in GenBank (Zhu et al., 2002). The analysis was run on 1000 pseudoreplicates. Bootstrap values of clades ≥70 are shown at the nodes. Numbers in brackets are corresponding to the individuals of *P. cattani* ($n = 32$) and *P. decipiens* sp. E ($n = 2$), showing identical ITS-1 and ITS-2 sequences. *Anisakis pegreffii* and *Ascaris suum* were used as outgroups.

Information on fish diet and habits were obtained from Cousseau and Perrotta (2004).

3. Results

3.1. Molecular identification of Pseudoterranova spp. larvae

The sequences analysis of the ITS region, including ITS1, 5.8S and ITS-2 of rDNA and the mtDNA *cox2* was completed on the same 34 specimens of *Pseudoterranova* spp. analyzed (Table 1). Alignments of ITS-1 (341 bp) and ITS-2 (299 bp) sequences identified in the present study in comparison with those deposited in GenBank for the species of the genus *Pseudoterranova* (only ITS-1 and ITS-2 of the rDNA were available) are presented as supplementary file.

Sequences of ITS-1 and ITS-2 rDNA from all the 32 *Pseudoterranova* specimens collected from the *O. flavescens* and the fish species *P. patagonicus*, *P. semifasciata* and *A. patachonicus* were identical, and matched 100% those sequences at the ITS-1 and ITS-2 of rDNA as deposited in GenBank for the species *P. cattani* by Zhu et al. (2002).

On the other hand, the two specimens of *Pseudoterranova* from *N. coriiceps* from Antarctica matched 100% the sequence at the mtDNA *cox2* gene of *P. decipiens* sp. E previously deposited in GenBank (Mattiucci *et al.*, 2013a); this finding has allowed identifying to the species level the larval anisakid from *N. coriiceps* as belonging to the taxon *P. decipiens* sp. E. Alignment of mtDNA *cox2* (519 bp) sequences of *P. cattani* and *P. decipiens* sp. E analyzed in the present study in comparison with the other species of *Pseudoterranova decipiens* complex previously characterized on the same gene and deposited in GeneBank (Mattiucci et al., 2013a) is presented as supplementary file.

The same specimens of *P. decipiens* sp. E from *N. coriiceps* were also sequenced at the ITS rDNA region; the two specimens have identical sequences at the ITS rDNA (GenBank accession number is given in Table 1).

The strict consensus of the Maximum Parsimony tree, inferred from the ITS-1 and ITS-2 sequences datasets depicted all the specimens of *P. cattani* studied ($n = 32$) as forming a unique phylogenetic lineage, well distinct from the other phylogenetic lineages formed by different species of the *P. decipiens* complex (Fig. 1). On the other hand, the specimens of *P. decipiens* sp. E from *N. coriiceps* have shown identical sequences and clustered in the same clade at the MP analysis, representing a distinct phylogenetic lineage from the other species of *Pseudoterranova* considered (Fig. 1).

The phylogenetic tree obtained by Bayesian analysis (BI) (Fig. 2), performed using $HKY + I + G$ (where $I = 0.307$ and $G = 0.173$) as the best fit substitution model – as obtained by jModeltest3.1 on the mtDNA *cox2* dataset – was in accordance with MP tree inferred from the ITS-1 and ITS-2 sequences data sets. All the larval specimens collected from *P. patagonicus*, *P. semifasciata* and *A. patachonicus* clustered together in clade composed also by the adult worms belonging to the species *P. cattani* collected from the

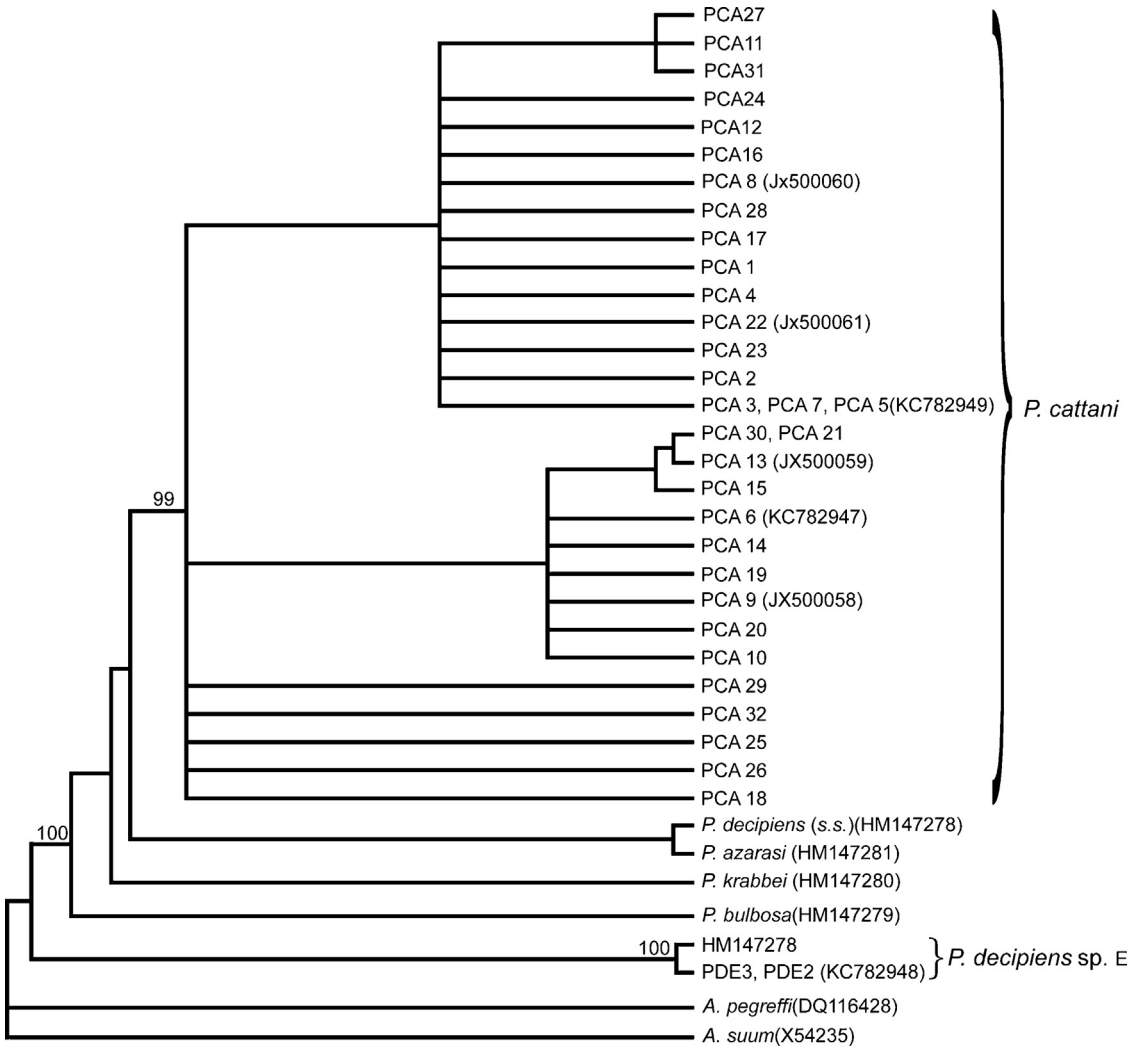

Fig. 2. Bayesian inference (BI) tree based on sequences of *P. cattani* and *P. decipiens* sp. E obtained at the mitochondrial *cox2* gene, performed by MrBayes3.1 (Huelsenbeck and Ronquist, 2005), using *HKY + I + G* model as selected by jModeltest (with Akaike Information Criterion (AIC)), where *I* = 0.307 and *G* = 0.173; the analysis used the MCMC algorithm: the number of chains was 4, the temperature of heated chains was 0.2, the number of generations was 1,000,000 and the sub-sampling frequency was 100, with a burn-in fraction of 0.25. The values of posterior probabilities (*Pp*) ≥95% (reported as percentage), indicative of significant support, are given at the nodes. Codes of the *Pseudoterranova* individuals are those reported in Table 1. In brackets the accession number of the specific individual of *Pseudoterranova* spp. studied in the present paper is reported. *Anisakis pegreffii* and *Ascaris suum* were used as outgroups.

individuals of *O. flavescens*, including those from Chile (Fig. 2). This clade received a high posterior probability support at the BI (Fig. 2); consequently, all these specimens were considered to belong to the species *P. cattani* (Fig. 2). In the BI analysis, the phylogenetic lineage represented by the species *P. cattani*, all larvae obtained from *P. semifasciata* clustered in the same sub-clade, whereas all adults from Chile clustered in the other sub-clade. Specimens from the other hosts were indistinctly distributed in both groups (Fig. 2).

At the same BI inference, the two larval specimens of *Pseudoterranova* collected from *N. coriiceps* of Antarctica clustered in a unique clade, having a high posterior probability support, together with the adult specimen of the Antarctic species *P. decipiens* sp. E collected from *Leptonychotes weddelli*, previously sequenced by us and available from GenBank (Fig. 2).

3.2. Description of third stage-larvae of Pseudoterranova cattani George-Nascimento et Urrutia, 2000

Medium sized and pale reddish nematodes, cuticle with inconspicuous transverse striations. Body reaching its greatest width near mid-body. Oral opening with three poorly developed lip primordia, one dorsal and two ventro-lateral, approximately equal in size. Dorsal lip primordia bearing a pair of lateral double papillae, ventro-lateral lip primordia each with lateral amphid and medio-lateral double papilla. Between ventro-lateral lips, a boring tooth is situated. Excretory pore opening beneath boring tooth. Excretory gland well developed, situated in the left ventrolateral region of the pseudocoel, extending beyond ventriculus along the anterior part of intestine. Deirids laterally located, posterior to nerve ring. Oesophagus long, slightly broader posteriorly than anteriorly. Long

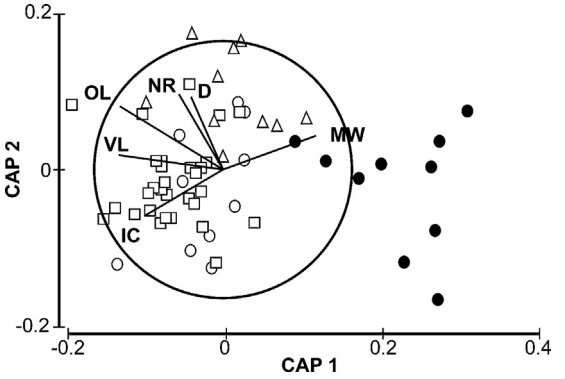

Fig. 3. Canonical analysis of principal coordinates (CAP) biplot of morphometric relationships in four samples third-stage larvae of *Pseudoterranova* spp. from four host species and two regions. The analysis was based on Euclidean distances calculated from untransformed data, and vector overlay are Spearman correlations of parasite species with the CAP axes (restricted to those having lengths > 0.5). Squares: worms from *Acanthisthius patachonicus*; triangles: worms from *Paralichthys patagonicus*; white circles: worms from *Pseudopercis semifasciata*, all from northern Argentine waters; black circles: worms from *Notothenia coriiceps* from Antarctica; D: deirid, IC: intestinal caecum; MW: maximum body width; NR: nerve ring; OL: oesophagus length; VL: ventriculus length.

ventriculus, slightly broader than oesophagus. Intestinal caecum dorsal to oesophagus, not reaching the oesophageal-ventricular junction. Short rectum, oblique in relation to anus and surrounded by three rectal glands (one ventral and two dorsal). Conical tail, bearing a small, elongated mucron. Measurements (mm) and morphometric relationships are given in Table 2.

3.3. Multivariate analysis of morphometric data sets

The CAP analysis showed significant differences between samples ($tr = 1.11$; $P = 0.0001$). The selected orthonormal PCO axes ($m = 3$), accounted for 94% of the variation in the data cloud, with a relatively low percentage of correct allocations (66.7%). The first canonical axes resulting from CAP analysis clearly separated Antarctic samples (Fig. 3), and strong support for the hypothesis of group differences was indicated by the reasonably large size of its canonical correlation ($\delta_1 = 0.89$), whereas the second canonical axis was less important ($\delta_2 = 0.57$). Indeed, samples from Argentine fish were distinguishable from Antarctic ones and were situated at the left of the CAP biplot, while those from Antarctic waters occupied the right side. Worms from *P. patagonicus* were separated from those of the other fishes from Argentina, mainly along the CAP 2. A wide body was the main characteristic of Antarctic larvae, whereas those from Argentine waters showed the highest values for most other relationships; the position of the nerve ring and deirids were responsible of the separation of worms from *P. patagonicus* along the second CAP axis.

Cross validation results showed that the lowest percentage of correctly allocated fish occurred for the samples from *A. patachonicus* and *P. semifasciata*, mostly due to cross assignation of nematodes between them. Conversely, the highest proportion of correctly allocated worms was observed for the Antarctic ones, with only one specimen

Table 2
Measurements (mm) and morphometric relationships of *Pseudoterranova* spp. third-stage larvae from *Acanthistius patachonicus*, *Pseudopercis semifasciata* and *Paralichthys patagonicus* from the South West Atlantic and *Notothenia coriiceps* from the Antarctic. Mean ± S.D. (range).

	Pseudoterranova cattani		*Pseudoterranova decipiens* sp. E	
Hosts	*A. patachonicus*	*P. semifasciata*	*P. patagonicus*	*N. coriiceps*
Number of specimens measured	30	10	10	10
Total length (TL)	26.85 ± 4.94 (17.16–35.74)	28.31 ± 2.87 (22.47–31.40)	24.25 ± 3.07 (20.20–30.10)	31.12 ± 4.48 (23.96–38.46)
Maximum width (MW)	0.67 ± 0.10 (0.40–0.90)	0.74 ± 0.07 (0.58–0.83)	0.68 ± 0.07 (0.59–0.78)	1.02 ± 0.11 (0.84–1.18)
Oesophagus length (OL)	1.89 ± 0.24 (1.48–2.34)	1.90 ± 0.14 (1.68–2.13)	1.76 ± 0.19 (1.54–2.08)	1.49 ± 0.21 (1.14–1.78)
Ventriculus length (VL)	1.07 ± 0.13 (0.70–1.30)	1.10 ± 0.14 (0.90–1.29)	0.95 ± 0.11 (0.80–1.20)	0.86 ± 0.10 (0.70–1.04)
Ventriculus width (VW)	0.25 ± 0.04 (0.18–0.30)	0.28 ± 0.03 (0.25–0.33)	0.23 ± 0.02 (0.20–0.26)	0.26 ± 0.03 (0.20–0.30)
Intestinal caecum length (IC)	0.94 ± 0.16 (0.62–1.22)	0.98 ± 0.18 (0.67–1.23)	0.69 ± 0.12 (0.50–0.80)	0.78 ± 0.11 (0.64–1.00)
Anterior end to nerve ring (NR)	0.37 ± 0.04 (0.30–0.44)	0.41 ± 0.03 (0.30–0.46)	0.42 ± 0.05 (0.35–0.52)	0.37 ± 0.02 (0.35–0.41)
Anterior end to deirids (D)	0.63 ± 0.07 (0.48–0.82)	0.67 ± 0.07 (0.54–0.75)	0.68 ± 0.09 (0.58–0.83)	0.64 ± 0.06 (0.54–0.73)
Tail length (T)	0.13 ± 0.02 (0.10–0.17)	0.14 ± 0.02 (0.11–0.18)	0.14 ± 0.02 (0.11–0.18)	0.14 ± 0.03 (0.10–0.19)
TL/MW	39.99 ± 3.68 (28.75–45.82)	38.63 ± 3.21 (32.03–41.87)	35.93 ± 3.07 (31.41–40.13)	30.48 ± 3.59 (21.78–34.48)
TL/OL	14.11 ± 1.47 (11.30–18.24)	14.90 ± 1.18 (13.42–16.84)	13.78 ± 1.38 (11.44–15.78)	21.50 ± 5.76 (15.48–32.05)
TL/VL	24.95 ± 2.56 (20.36–30.14)	25.90 ± 2.94 (20.43–30.08)	25.54 ± 2.68 (22.89–30.50)	36.34 ± 6.17 (28.93–47.29)
TL/VW	109.37 ± 17.10 (70.77–137.54)	100.30 ± 12.33 (80.61–124.80)	106.89 ± 13.36 (85.83–129.09)	121.04 ± 20.35 (79.87–147.92)
TL/IC	28.59 ± 3.75 (21.58–41.32)	29.82 ± 5.68 (21.61–40.41)	35.96 ± 9.09 (25.75–53.75)	40.53 ± 7.10 (31.86–50.56)
TL/D	42.87 ± 6.47 (32.86–55.06)	42.70 ± 4.03 (34.17–50.47)	35.32 ± 1.24 (32.84–36.86)	49.36 ± 10.30 (34.42–68.68)
TL/NR	71.28 ± 9.60 (52.32–94.22)	69.13 ± 2.71 (64.06–72.56)	58.44 ± 6.39 (46.98–66.70)	82.50 ± 11.87 (62.40–97.17)
TL/T	212.68 ± 36.74 (136.90–309.44)	199.58 ± 32.71 (149.83–241.54)	184.50 ± 38.94 (117.71–240.80)	228.80 ± 60.13 (159.29–331.62)

Table 3
Cross validation of CAP analyses (leave-one-out allocation of individual *Pseudoterranova* spp. larvae from four fish species and two regions).

Origin group	Classified to				% Correct
	A. patachonicus	*P. semifasciata*	*P. patagonicus*	*N. coriiceps*	
A. patachonicus	18	9	3	0	60
P. semifasciata	3	5	2	0	50
P. patagonicus	1	1	8	0	80
N. coriiceps	0	0	1	9	90

(10%) wrongly allocated to *P. patagonicus*; this nematode was the smallest in the sample. On the other hand, 100% of worms from North Argentine waters were correctly allocated to fish from this zone (Table 3).

PERMANOVA results on the four samples detected significant variability across samples from different hosts (*Pseudo* $F = 17.22$; $P < 0.0001$). Pair-wise tests between samples showed that there were significant differences between those comparisons involving worms from Antarctic (all $P < 0.0001$). Among the Argentine samples, worms from *P. patagonicus* also differed from those of the other two host species (both $P < 0.01$), which were indistinguishable each other ($P > 0.05$).

3.4. Prevalence and intensity of infection

Third stage larvae of *Pseudoterranova* were found free or encapsulated in the coelomatic cavity, mesenteries, liver and muscle of fish (Table 4). Only *P. semifasciata* showed no infection in the muscle, but showed the highest prevalence, whereas *P. patagonicus* harboured larvae mainly in the muscle, but with lower prevalence and intensity values.

Larvae of *Pseudoterranova* were uncommon in the fish species studied in the Bonaerense region, with only 27% of host species infested, reaching high values of prevalence only in *P. patagonicus*, *A. patachonicus* and *P. semifasciata* and high mean abundance in the latter two species (Table 5). Parasites were present in fish species of medium to large size (about 30 cm or more) and at high trophic level (over 3.7, excepting *X. rasile*).

4. Discussion

As a consequence of their implications on public health, anisakids play a central role in discussions on marine fish nematodes (Margolis, 1977; Smith and Wootten, 1978; Smith, 1983, 1999; Berland and Fagerholm, 1994; Mattiucci et al., 2011, 2013a,b). However, their identification is still problematic, particularly for the larval stages (Berland and Fagerholm, 1994), including those of *Pseudoterranova* spp. Records of these parasites in fishes inhabiting the Argentine Sea are still scarce and in part erroneous, with misidentification of innocuous species as pathogenic and *vice versa* (Timi et al., 2001) and there is still a need for information on the identity, microhabitat, distribution and abundance of the nematodes of the genus *Pseudoterranova* in these fish as well as in others that are harvested for human consumption. The importance of such information also relies in the costs that the presence of anisakids implies for the fishery industry, reducing both the quality and the market value of the products. The present findings indicate that members of the genus *Pseudoterranova* in the studied areas of Pacific and Atlantic coasts of South America are representatives of a unique species, namely *P. cattani*, whose distribution mirrors that of its definitive host, *O. flavescens*.

Pseudoterranova cattani has been recently recorded in *O. flavescens* from the Patagonia, Argentina (Hernández-Orts et al. (2012)). Unfortunately, the stage of preservation of adult specimens found in *O. flavescens* from Quequén, which had the cuticle mostly degraded and detached, did not allow a proper morphologic identification at the species level. Similarly, most specimens found by Hernández-Orts et al. (2012) were in the same condition.

Adult worms from Chilean and Argentine waters and larval specimens from Argentine fish had ITS-1 and ITS-2 sequences that were identical each other and with respect to the sequences of *P. cattani* previously deposited (Zhu et al., 2002). Furthermore, the present paper provides the first molecular characterization of *P. cattani* at the adult stage based on mtDNA *cox2* sequences analysis and the unequivocal identification of larval *P. cattani* from fish of commercial importance by using genetic/molecular methodology.

Both the MP and BI phylogenetic trees inferred from the ITS-1 and ITS-2 of rDNA sequences data sets and those from mtDNA *cox2* had the similar topology in showing both *P. cattani* and *P. decipiens* sp. E as forming two distinct phylogenetic lineages, corresponding to two distinct biological species. Both at the nuclear (ITS) and mitochondrial (mtDNA *cox2*) level, the species *P. decipiens* sp. E appears to be the sister taxon with the respect to the remaining species of *Pseudoterranova* (Figs. 1 and 2). Moreover, the phylogenetic trees based on mtDNA *cox2* sequences yielded a close relationship between *P. decipiens* (*s.s.*) and *P. azarasi* in accordance with Nadler et al. (2005) based on ITS sequences analysis.

Table 4
Prevalence and mean intensity of larval *Pseudoterranova* in three fish species from the Bonaerense region of the Argentine Sea.

Host species	All organs		Muscle		Source
	P	MI	*P*	MI	
A. patachonicus	57.8	25.5 ± 59.2	42.2	7.4 ± 8.8	Present study
P. semifasciata	93.3	17.7 ± 17.1	0	0	Timi and Lanfranchi (2009a)
P. patagonicus	17.6	1.4 ± 0.7	15.7	1.2 ± 0.5	Alarcos and Timi (2012)

Table 5
Fish species studied in northern Argentine coasts (Buenos Aires Province): size, trophic level, habitat, diet and infestation parameters of *Pseudoterranova cattani*.

Host	*N*	Total length (cm) ± S.D.	Trophic level	Habitat	Main prey item	Prevalence	Mean abundance	Source
Clupeiformes: Engrauliidae								
Anchoa marinii Hildebrand, 1943	136	9.8 ± 0.9	3.4	Pelagic	Plankton	0	0	1
Engraulis anchoita Hubbs et Marini, 1935	2045	9.8 ± 4.9	2.51	Pelagic	Plankton	0	0	2
Atheriniformes: Atherinopsidae								
Odontesthes incisa (Jenyns, 1829)	100	8.9 ± 0.7	3.29	Pelagic	Plankton	0	0	1
Odontesthes argentinensis (Valenciennes, 1835)	69	28.5 ± 5.5	3.33	Pelagic	Unknown	0	0	1
Odontesthes smitti (Lahille, 1929)	59	25.7 ± 1.6	3.30	Pelagic	Plankton	0	0	3
Anguilliformes: Congridae								
Conger orbignianus Valenciennes, 1847	50	80.69 ± 12.86	3.72	Benthic/demersal	Fish	2.0	0.02	4
Scorpaeniformes: Triglidae								
Prionotus nudigula Ginsburg, 1950	101	19.7 ± 3.0	3.77	Benthic	Invertebrates	0	0	5
Gadiformes: Merluciidae								
Merluccius hubbsi Marini, 1933	66	40.5 ± 5.5	4.23	Demersal	Plankton/fish	0	0	6
Perciformes:								
Carangidae								
Trachurus lathami Nichols, 1920	223	18.8 ± 0.8	3.99	Pelagic	Plankton	0	0	7
Mullidae								
Mullus argentinae Hubbs et Marini, 1933	75	19.5 ± 1.9	3.45	Benthic	Invertebrates	0	0	8
Latridae								
Nemadactylus bergi (Norman, 1937)	100	34.5 ± 3.2	3.45	Benthic	Invertebrates	0	0	9
Sparidae								
Pagrus pagrus (Linnaeus, 1758)	123	29.8 ± 4.0	3.55	Demersal	Invertebrates/fish	0	0	1
Percophidae								
Percophis brasiliensis Quoy et Gaimard, 1825	195	55.9 ± 5.9	4.33	Demersal	Fish	0	0	10
Pinguipedidae								
Pinguipes brasilianus Cuvier, 1829	210	33.4 ± 3.8	3.80	Demersal	Invertebrates	0	0	11
Pseudopercis semifasciata (Cuvier, 1829)	30	71.2 ± 3.5	3.88	Demersal	Invertebrates/fish	93.3	17.7	12
Sciaenidae								
Cynoscion guatucupa (Cuvier, 1830)	188	45.4 ± 5.9	4.23	Demersal	Fish	0	0	13
Serranidae								
Acanthistius patachonicus (Jenyns, 1842)	45	35.6 ± 6.4	4.01	Demersal	Invertebrates/fish	57.8	25.5	1
Pleuronectiformes: Paralichthyidae								
Xystreuris rasile (Jordan, 1891)	48	29.3 ± 2.6	3.29	Benthic	Invertebrates	2.1	0.1	14
Paralichthys isosceles Jordan, 1891	51	27.9 ± 2.1	4.04	Benthic	Invertebrates/fish	2.0	0.02	14
Paralichthys patagonicus Jordan, 1889	51	35.20 ± 2.64	3.90	Benthic	Invertebrates/fish	17.6	1.4	14
Ophidiiformes: Ophiididae								
Genypterus blacodes (Forster, 1801)	27	46.0 ± 4.8	4.18	Demersal	Fish	0	0	15
Raneya brasiliensis (Kaup, 1856)	76	23.7 ± 2.3	3.57	Benthic	Invertebrates	0	0	16

(1) Present study; (2) Timi and Poulin (2003); (3) Carballo et al. (2012); (4) Timi and Lanfranchi (2012); (5) Timi and Lanfranchi (2009b); (6) Sardella and Timi (2004); (7) Braicovich et al. (2012); (8) Lanfranchi et al. (2009); (9) Rossin and Timi (2010); (10) Braicovich and Timi (2010); (11) Timi et al. (2009); (12) Timi and Lanfranchi (2009a); (13) Timi et al. (2005); (14) Alarcos and Timi (2012); (15) Sardella et al. (1998); and (16) Vales et al. (2011).

While at the ITS-1 and ITS-2 genes no genetic variation was observed at the intraspecific level in *P. cattani*, the mitochondrial DNA *cox2* gene arrangement has proved to be highly polymorphic in that species. This finding supports the possible use of this gene locus in further studies of population genetic structures and phylogeography of this species, as recently suggested for other anisakid species (Baldwin et al., 2011). In the case of the mtDNA *cox2* gene, nucleotide variation was mainly related to changes in the position of the third codon, which is consistent with that previously observed in species of *Anisakis* (Valentini et al., 2006) and *Contracaecum* (Mattiucci et al., 2008; Garbin et al., 2011). Moreover, the possible use of mtDNA *cox2* gene sequencing of a large number of these parasites could give, in future applications, information about the possible existence of population structure of their definitive hosts, the South American sea lion *O. flavescens*, as confirmed in other studies (Túnez et al., 2007, 2010; Feijoo et al., 2011) and/or their intermediate/paratenic hosts from different fishing areas of the Chile and Argentine waters.

Although morphologic/morphometric diagnostic characters to discriminate between males of the species of the *P. decipiens* (*s.l.*) complex have been achieved (Paggi et al., 1991, 2000; Di Deco et al., 1994; Mattiucci et al., 1998; George-Nascimento and Urrutia, 2000), their larval stages have been largely considered as indistinguishable. Larvae of *P. cattani* harboured by *A. patachonicus* and *P. semifasciata* from Argentina are morphologically indistinguishable and clearly different from those of *P. decipiens* sp. E, and, at a lesser extent, from those of *P. cattani* collected in the fish *P. patagonicus*, as demonstrated by multivariate analysis. This finding could imply that larval forms within the species complex could be identified based on their morphology/morphometry. However significant morphometric and allozyme variability in *P. cattani* was found in larvae from sympatric fish species from Chile (George-Nascimento and Llanos, 1995). These authors attributed this variability to selective pressures arising from the internal environment of intermediate hosts, which could explain the morphologic differences between larvae from flounders in relation to those parasitizing *A. patachonicus* and *P. semifasciata*. Comparative studies, including samples of the other taxa of the *P. decipiens* species complex from different regions and fish hosts are needed to determine the validity of larval morphometry in distinguishing among sibling species, or at least between some of them.

Pseudoterranova cattani is a common and abundant parasite in a variety of fish species from Chile (George-Nascimento and Llanos, 1995), where larvae have been identified as either *P. cattani*, *P. decipiens* (*s.l.*) or *Pseudoterranova* sp. (Muñoz and Olmos, 2008). A contrasting situation occurs in northern Argentine waters, where most fish species so far investigated are free of these parasites, and those harbouring larvae show, in general, low levels of parasitism. Furthermore, some of the previous records of *Pseudoterranova* spp. in this region are erroneous or need validation. In fact, their records in *M. hubbsi* and *Porichthys porosissimus* by Sardella and Timi (1996) and Tanzola et al. (1997), respectively, are actually members of the genus *Terranova* (Sardella and Timi, 2004; Tanzola and Guagliardo, 2004), and it is likely that other reports also concern this genus, especially those from the pelagic *Scomber japonicus* (Cremonte and Sardella, 1997), since the life cycle of *P. decipiens* (*s.l.*) occurs commonly in demersal and benthic fishes (Margolis, 1977; Marcogliese, 1996).

Despite the observed rarity of larval *Pseudoterranova* among Argentine fishes, their distribution is not uniform along coastal waters. In the southern region (Patagonia), larvae are more common and have been reported in fishes that are free of these parasites in northern waters (Reimer and Jessen, 1981, as *Phocanema* sp.; Sardella and Timi, 2004; Herreras et al., 2000; Sardella et al., 1998; Timi et al., 2009; Vales et al., 2011), as well as in other species caught in Patagonia (Gayevskaya et al., 1990; Sardella et al., 1998; Carballo et al., 2011) and squids (as *Porrocaecum* sp.) (Nigmatullin, 1989; Nigmatullin and Shukhgálter, 1990). The latter records are probably misidentifications, because larvae are described as having the excretory pore at level of the nerve ring (Nigmatullin and Shukhgálter, 1990).

The distribution of definitive hosts has been postulated as one of the most important biotic factors determining the distribution of *P. decipiens* (*s.l.*) (McClelland, 2002). *O. flavescens* is distributed along a broad latitudinal range along the South American coastline, from Peru in the Pacific to Brazil in the Atlantic (Vaz Ferreira, 1982). This species is apparently the only suitable definitive host for *P. cattani* (George-Nascimento and Llanos, 1995). Indeed, the South American fur seal *Arctocephalus australis*, sympatric with the sea lions in Uruguay and in some localities of the Argentine coasts, has been reported as harbouring only larval stages in Patagonia (Hernández-Orts et al., 2012).

The proximity to colonies of definitive hosts is one of the causes that increase the levels of parasitism by *Pseudoterranova* spp. in fish (Jensen and Idås, 1992; Des Clers and Anderson, 1995). While large colonies of *O. flavescens* are established in the southern coasts of South America (Suárez et al., 2005; Sepúlveda et al., 2011), there is a gap of 1000 km between Patagonia and Uruguay that corresponds mainly to the coast of the Buenos Aires province, where only small non reproductive colonies are found at Mar del Plata and Quequén harbours (Suárez et al., 2005) composed of approximately 800 and 100 males, respectively (Túnez et al., 2007), and a semi-permanent settlement at Isla Trinidad, Bahía Blanca estuary, composed by 0–150 individuals, depending on the season (Petracci et al., 2010). As shown for *P. decipiens* in the Northern Hemisphere (Marcogliese, 1995; Boily and Marcogliese, 1995), the lower densities of sea lions in Bonaerense coasts could account for the low densities of *P. cattani* in fishes, especially in southern Bonaerense coasts (Bahía Blanca region), where only one (*A. patachonicus*) out or thirteen bony fish species has been reported harbouring low numbers of worms (Tanzola and Guagliardo, 2004).

It is likely that the lower densities of sea lions in the northern Argentine coasts sustain a small population of *P. cattani*. Although there are no previous data on parasite burdens in sea lions of this zone, it should be noted that in Uruguayan coasts *P. cattani* is also scarce. In fact, adult *O. flavescens* examined in this locality showed low values of prevalence (44%) and abundance (1.89 ± 1.23) of larval worms (George-Nascimento, 1991), values similar to those found in the present study in Bonaerense

coasts, but lower than those found in Patagonian coasts. For example Berón-Vera et al. (2005) reported a prevalence of 63.63% and 22.2% and a mean abundance of 317.81 and 5.6, for male and female hosts, respectively; more recently Hernández-Orts et al. (2012) observed a prevalence of 66.1% and a mean intensity of 14.8 worms in a sample of 56 sea lions from the same region. Similarly, higher levels of parasitism have been recorded in *O. flavescens* in Chile (prevalence = 100%, mean abundance = 131.1 ± 125.5) (George-Nascimento, 1991).

However, because of the reasonably large colony of *O. flavescens* inhabiting Uruguayan coasts, causes other than definitive host density could determine the population size of this parasite in Uruguayan and northern Argentine waters. The distribution patterns of marine parasites are mainly determined by temperature-salinity profiles and their association with specific masses of water (Esch and Fernández, 1993), therefore their abundance could be influenced by the characteristics of the local ecosystem and its trophic web (Marcogliese, 2001, 2002; Mattiucci and Nascetti, 2008). Water temperature is considered one of the most important abiotic factors determining the distribution of *P. decipiens s.l.* (McClelland, 2002). Low water temperatures are a shared feature of both Chilean and Patagonian coasts (Acha et al., 2004; Rivas et al., 2006). On the other hand, the continental shelf of the Buenos Aires Province is characterized by higher water temperatures, where also salinity is reduced by the discharge of freshwater (Jaureguízar et al., 2006; Acha et al., 2008). Higher temperatures in this region could be suboptimal for the completion of the life cycle of the parasites, accounting for their low densities in fish and mammal hosts. In fact it has been observed that ova of *P. decipiens* fail to complete development and hatch at temperatures approaching 25 °C (McClelland, 1982). Furthermore, environmental conditions can also affect the distribution and/or abundance of suitable previous invertebrate hosts, still unknown for *P. cattani*.

The comparative analysis of host traits shows that a large size, high trophic level and benthophagic habits seem to be the combination of host features that favours the transmission of *P. cattani* in this region. These features agree with those reported for fish acting as secondary fish hosts for *P. decipiens* (see McClelland, 2002 and references therein), which are large demersal piscivores, acquiring parasites by preying on smaller fish (primary fish hosts), which in turn are benthic consumers that acquire the parasite directly from invertebrate hosts. This parasite is absent in the probable "primary fish hosts" (small benthic species) so far studied in Bonaerense waters, including *Raneya brasiliensis* and *Cynoscion guatucupa*, two of the main preys of *O. flavescens* in Bonaerense coasts (Suárez et al., 2005), the third species in order of importance among dietary items being *Engraulis anchoita.* These anchovies, due to their planktophagus diet, are free of larval *P. cattani* (Timi, 2003; Timi and Poulin, 2003). Similarly, sea lions in Uruguay feed mainly on the engraulid *Anchoa marinii* (Naya et al., 2000), a species found to be free of *P. cattani* in the present study. On the other hand, although *P. patagonicus*, *P. semifasciata* and *A. patagonicus* are consumed by sea lions, the size of these fish as adults is in general out of the range of prey sizes eaten by *O. flavescens* (Koen Alonso et al., 2000; Suárez et al., 2005; Riet-Sapriza et al., 2013). Larger fish, and therefore those harbouring the highest numbers of parasites, do not contribute to the completion of the life cycle of *P. cattani* in the area.

A combination of factors seems to drive the population size of *P. cattani* in the northern boundary of distribution of *O. flavescens* in the Atlantic, including the environmental conditions (warm waters with low salinity), the density and dietary preferences (or prey availability) of definitive hosts and the life-cycle pathways of the parasite.

The musculature of the studied fish species was in general free of this parasite, the exception being *A. brasilianus* and *P. patagonicus*, both with relatively high parasite burdens. Consequently, these fish species constitute the most potentially hazardous ones for human health among the host species herein studied. Although the recognition of this nematode at specific level does not imply changes in the problems it can cause for the fishery industry, it can have implications from a public health perspective. Indeed, and because of the scattered distributions of *Pseudoterranova* spp. and their geographic patterns of pathogenicity, it has been suggested that their pathological effects in humans could differ among worm species (Arizono et al., 2011; Mattiucci et al., 2013b). In fact, some regionality has been found in the degree of pathology caused by larval *Pseudoterranova*. In Japan, most patients have severe pathology caused by penetration of the alimentary tract, whereas most cases diagnosed in Europe and Chile have been classified as "transient luminal" and asymptomatic, with worms being expelled by coughing, vomiting or defecation (Smith, 1999; McClelland, 2002; Torres et al., 2007). The aethiologic agent of pseudoterranovosis has been recently identified in Japan as *P. azarasi* (Arizono et al., 2011), whereas only *P. cattani* has been identified at species level for both larval and adult forms infecting fish and sea lions in Chile (George-Nascimento and Urrutia, 2000, present study). Consequently, although no human cases have been reported in Argentina, the risk for human health should be expected to be similar to that recorded in Chilean cases. On the other hand, the absence of human cases in Argentina is likely due to the fact that the culinary tradition involves mainly well cooked fish.

Conflict of interest statement

No financial or personal relationships are maintained with other people or organizations that could inappropriately influence or bias this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2013.09.033.

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