FEATURE ARTICLE

The *Anisakis simplex* complex off the South Shetland Islands (Antarctica): endemic populations versus introduction through migratory hosts

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ABSTRACT: Third-stage larvae (L3) of 2 *Anisakis* nematode species, *A. simplex* C and *A. pegreffii* (both *A. simplex* sibling species complex), were isolated from migrating myctophids around the South Shetland Islands. *Gymnoscopelus nicholsi* were parasitized by both nematode species, at a prevalence of 22.7% for *A. simplex* C and 4.0% for *A. pegreffii*, while *Electrona carlsbergi* harbored *A. simplex* C at a prevalence of 9.1%. The ITS-1, 5.8S, and ITS-2 regions of the nematodes were identical to specimens from Pacific Canada or California (*A. simplex* C) and the coast of China (*A. pegreffii*), confirming an extensive range of distribution for both species. The occurrence in migrating myctophids coupled with rare findings from other teleosts leads to the conclusion that both species were introduced from outside the Antarctic. Consequently, they are at their most southern range in the Southern Ocean, and an earlier molecular record of *A. simplex* C from the elephant seal *Mirounga leonina* may have detected an accidental case of infection. Delphinids, which frequently occur along South America but not in the Southern Ocean, are suggested as being the typical final hosts in the life cycles of *A. simplex* C and *A. pegreffii*. The myctophids had only few prey items within their stomachs, dominated by euphausiids, including *Euphausia superba* and planktonic copepods. These are common intermediate hosts for anisakid nematodes and are an important component of the Antarctic food web. Regular introduction events through migrating whales and myctophids are considered responsible for the occurrence of *Anisakis* infection in the high Antarctic.

KEY WORDS: *Anisakis simplex* C · *Anisakis pegreffii* · South Shetland Islands · Southern Ocean (Antarctica) · Myctophids · Sibling species · Zoogeography

INTRODUCTION

Antarctica (including the surrounding Southern Ocean) offers a unique natural laboratory to study the relationships of the evolutionary processes and species adaptation to extreme climate and environmental conditions (Clarke et al. 2007). The low water temperatures, the missing coastal zone due to the shelf-ice
cover, and the drifting icebergs that regularly strand and affect the benthic communities, are some of the unique features that necessitate special adaptations of the flora and fauna. Another unique characteristic is the missing strict separation between the continental shelf and the deep sea, enabling deep-sea species to also occur in shallower waters, and especially benthic-demersal shallow water species to extend their range into the deep sea.

A species-rich but well hidden component of the Antarctic fauna, fish parasites have been studied since the 19th century, with early comprehensive studies such as by Fuhrmann (1921) or Johnston (1937). Most studies have focused on descriptions of new species and the faunistic composition of parasitic helminths (e.g. Digenea: Zditzowiecki 1991, 1997; Cestoda: Rocka 2006; Nematoda: Klöser et al. 1992, Palm et al. 1994; Acanthocephala: Zditzowiecki 1996). Although they are highly abundant in most studied fish species, the species richness of parasitic nematodes in the Southern Ocean seems to be limited in comparison to other parasite groups. Only few nematodes of unknown life cycles such as Ascarophis and Paraniaskiops mature in Antarctic fish. More represented are genera that utilize fish as second intermediate/paratenic hosts and birds/pinnipeds as final hosts, such as Contracaecum and Pseudoterranova (e.g. Palm 1999, Rocka 2004, 2006).

Most Antarctic fish belong to the perciform suborder Notothenioidei, especially the families Nototheniidae and Channichthyidae. Both have been the focus of investigations (e.g. distribution, growth, parasite fauna) and represent the most diverse and abundant fish taxa along the Antarctic coast and continental shelf (e.g. Zditzowiecki 1991, 1997). A minor part of the known fish species in the Southern Ocean, including endemic and migrating myctophids, live in the meso- and bathypelagic zone. Especially species of the genera Electrona and Gymnoscopelus occur on the outer shelf, but they reach their maximum abundance in terms of biomass and diversity in the pelagic ecosystem. Besides consuming the krill Euphausia superba, Myctophidae play a significant role as consumers of zooplankton and they are an important food source for predators of higher trophic levels in the Antarctic food web (e.g. Pusch et al. 2004). Parasitological studies of fish species from the Antarctic continental slope and the deep sea are scarce (e.g. Walter et al. 2002, Palm et al. 2007). Rocka (2006) summarized the available information on the life cycle biology, specificity, and geographical distribution of the Digenea, Cestoda, Nematoda, and Acanthocephala of Antarctic bony fishes. The author stated that almost all helminth species maturing in Antarctic bony fishes are endemic, whereas only few are cosmopolitan or bipolar. Specificity to intermediate or paratenic hosts is wide for the majority of Antarctic helminths, while that for the final host is often narrower (Rocka 2006).

The life cycles of nematodes parasitizing marine mammals differ in the use of pelagic or benthic invertebrates (mainly crustaceans) as their first intermediate hosts and several fish species as the second or paratenic hosts (e.g. Palm et al. 1994, 2007, Palm 1999, Klösmal et al. 2008b). Initial studies on the life cycle biology of anisakid nematodes in the Weddell Sea identified a pelagic life cycle for Contracaecum radiatum (Klöser et al. 1992, Klöser & Plötz 1992) and a benthic cycle for C. osculatum and Pseudoterranova decipiens (Palm et al. 1994, Palm 1999). Since these nematodes colonised the Antarctic environment (after establishment of the Antarctic Circumpolar Current [ACC] about 25 to 22 million years ago), they have largely maintained a similar life cycle biology to that of their relatives from non-Antarctic waters such as in the North Atlantic. Another marine nematode, the whaleworm Anisakis spp., is rare in the high Antarctic, although this genus is common in tropical and boreal regions and also in the deep sea (e.g. Klimpel et al. 2006, 2008b, Mattiucci & Nascetti 2008, Rokicki et al. 2009).

Recent molecular studies identified distinct anisakid species within the Antarctic that were morphologically very similar to but genetically different from their non-Antarctic counterparts. To date, the sibling species Pseudoterranova decipiens E, Contracaecum osculatum D, and C. osculatum E (e.g. Zhu et al. 2002, Mattiucci & Nascetti 2008) have been reported from this region. The record of Anisakis simplex C from the sub-Antarctic (Mattiucci & Nascetti 2008), Anisakis sp. on the sub-Antarctic islands (e.g. Rocka 2004, 2006, Brickle et al. 2005), and the high abundance of whales led to the assumption that this genus also occurs in the Antarctic. However, a series of parasitological studies of notothenioid fish could either not detect this parasite or found it with extremely low infestation rates (e.g. Palm et al. 1998, 2007, Zditzowiecki & Laskowski 2004). In contrast, Rokicki et al. (2009) recorded unidentified Anisakis sp. from the same fish species with higher infestation rates.

During our investigation of 3 myctophid fish species (Electrona antarctica, E. carlsbergi, Gymnoscopelus nicholsi) off the South Shetland Islands, we were able to isolate and genetically identify Anisakis sibling species. Here we compared these nematodes to specimens from other regions in order to understand this unusual restricted occurrence in myctophid fish and its means of transmission. Possible reasons for the rareness of Anisakis sibling species in most Antarctic fish that have been studied thus far are discussed. We used the endemic E. antarctica and the migrating, non-endemic E. carlsbergi and G. nicholsi because they play an important role in the Antarctic food web.
MATERIALS AND METHODS

Sample collection. Fish were sampled in December 2006 on board the German RV ‘Polarstern’ (research cruise ANT XXII/8) during the field phase of the international project ‘Convention for the Conservation of Antarctic Marine Living Resources’ (CCAMLR) to Elephant Island (South Shetland Islands). Sampling was conducted with a bottom trawl at a trawling speed between 3.3 and 4.1 knots and a towing time of 30 min. In total, 75 specimens of Gymnoscopelus nicholsi were captured at Stn 637 (position 61° 5.67’ to 61° 5.92’ S, 56° 10.00’ to 56° 6.43’ W; trawling depth range 425 to 357 m, mean depth 391 m; Fig. 1, AI-2), 50 specimens of Electrona antarctica at Stn 616 (60° 49.81’ to 60° 49.20’ S, 55° 36.76’ to 55° 40.27’ W; trawling depth range 483 to 486 m, mean depth 484 m), and 55 specimens of E. carlsbergi at Stn 622 (60° 56.70’ to 60° 55.93’ S, 55° 52.71’ to 55° 50.79’ W; trawling depth range 218 to 307 m, mean depth 262 m; Fig. 1, AI-1; all Family Myctophidae) in order to study their metazoan parasites and stomach contents. All fish were deep frozen at –40°C immediately after capture for subsequent examination. In the ship’s laboratory, the standard length (SL, to the nearest 0.1 cm) and total weight (TW, to the nearest 0.1 g) were determined (Table 1). Prior to examination, each specimen was defrosted at 0 to 1°C. Fish were identified in accordance with Gon & Heemstra (1990).

Stomach content analyses. The stomach contents were sorted, and prey items were identified to the lowest possible taxon and grouped into taxonomic categories. To determine the relative importance of food items, the numerical percentage of prey (N%), the weight percentage of prey (W%), and the frequency of occurrence (F%) were determined (Hyslop 1980, Amundsen et al. 1996). Using these 3 indices, an index of relative importance (IRI; Pinkas et al. 1971) was calculated. The importance of a specific prey item increases with higher values for N, W, F, and IRI.

Parasitological examination. The presence of anisakid nematodes was determined for all organs using a stereomicroscope. The body cavity was opened to microscopically examine the liver, stomach, pyloric caeca, intestine, and gonads for nematodes, and the stomach contents were removed. The specimens were freed from host tissue and morphologically identified using existing keys and descriptions. After removal from the host tissue, Anisakis spp. larvae were stored in absolute (~100.0%) ethanol for further molecular identification (see below). The ecological and parasitological terminology follows Bush et al. (1997):

Fig. 1. Areas of investigation. AI-1: area of investigation 1, Electrona antarctica and E. carlsbergi; AI-2: area of investigation 2, Gymnoscopelus nicholsi
Infection (P) is the number of infected fish with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined (expressed as a percentage). Intensity (of infection, I) is the number of individuals of a particular parasite species in a single infected host (expressed as a numerical range). Mean intensity (of infection, mI) is the average intensity; in other words, it is the total number of parasites of a particular species found in a sample divided by the number of infected hosts. Mean abundance (A) is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined, including both infected and uninfected hosts. Furthermore, we used the following terms and definitions: final host – host in which a parasite reaches sexual maturity; intermediate host – required by a parasite to complete its life cycle (usually the host in which it undergoes considerable morphological or physiological change); paratenic host – not required by a parasite to complete its life cycle, and no detectable morphological change occurs within this host.

**Table 1.** Number, lengths, weights, food items, and parasites of the studied fish species. F%: frequency of occurrence, N%: numerical percentage of prey, W%: weight percentage of prey, IRI: index of relative importance; P%: prevalence; I: intensity; mI: mean intensity; A: mean abundance. See ‘Materials and methods’ for definitions of terms.

<table>
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<tr>
<th>Fish species</th>
<th>Gymnoscopelus nicholsi (n = 75)</th>
<th>Electrona carlsbergi (n = 55)</th>
<th>Electrona antarctica (n = 50)</th>
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<td><strong>Range</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Range</strong></td>
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<tr>
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<td>4.3–7.9</td>
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<td><strong>F%</strong></td>
<td><strong>N%</strong></td>
<td><strong>W%</strong></td>
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<td>Gammaridae</td>
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<td>Gastropoda</td>
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<td>Teleostei undet.</td>
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<td>0.5</td>
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<td><strong>I</strong></td>
<td><strong>ml</strong></td>
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<td>Anisakis pegreffii</td>
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PCR amplification and sequencing of ITS-1, 5.8S, and ITS-2. Genomic DNA was isolated and purified from individual *Anisakis* spp. larvae using a genomic DNA extraction kit (Peqlab Biotechnology) according to the instructions of the manufacturer. The rDNA region comprising the internal transcribed spacer ITS-1, 5.8S, ITS-2, and flanking sequences (=ITS+) was amplified using the previously described primers NC5 (5’-TTA GGT TCT TTT CCT CCG CT-3’) and NC2 (5’-TTA GGT TCT TTT CCT CCG CT-3’) (Zhu et al. 2000). The PCR reaction (26 µl) included 13 µl of Master-Mix (Peqlab Biotechnology) containing dNTPs, MgCl₂ buffer, and *Taq* polymerase, 3 µl of each primer, 2 µl of distilled Water, and 5 µl of genomic DNA. Each PCR reaction was performed in a thermocycler (Biometra) under the following conditions: after initial denaturation at 95°C for 15 min, 30 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), and 72°C for 1 min (extension), followed by a final extension at 72°C for 5 min. Samples without DNA were included in each PCR run. PCR products were examined on 1% agarose gels. A 100 bp ladder marker (peqGOLD) was used to estimate the size of the PCR products. To identify the anisakid nematodes, the PCR products were purified with an E.Z.N.A. Cycle-Pure Kit (Peqlab Biotechnology). Afterwards, a total volume of 7 µl, including 2 µl of primer (individually) and 5 µl of the PCR product (250 ng µl⁻¹) were sequenced by Seqlab (Göttingen, Germany). Both spacers and the 5.8S gene from each PCR product were sequenced in both directions, using primers NC5, NC13 (forward; 5’-ATC GAT GAA GAA CGC AGC-3’), NC13R (reverse; 5’-GCT GCG TTC TTC ATC GAT-3’), XZ1R (reverse; 5’-GGA ATG AAC CCG ATG GCG CAA T-3’), and NC2. The obtained sequences were identified via GenBank and aligned with previously characterized sequences of anisakid nematodes, using CLUSTAL W (1.83) Multiple Sequence Alignments (Thompson et al. 1994). Among the observed *Anisakis* siblings, the nucleotide sequences of all 3 regions...
Table 2. Anisakis spp. and Hysterothylacium aduncum. GenBank accession numbers of sequences of ascaridoid nematodes used for comparative analyses (Fig. 2). F: fish host, W: whale host, S: seal host, A: adult, L: larva, s.s.: sensu stricto

<table>
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<th>Species</th>
<th>Code</th>
<th>Host</th>
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<th>A/L</th>
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*Sequences from the present study

(ITS-1, 5.8S, ITS-2) were compared. Resulting sequence data of anisakid siblings were compared to previously published sequence data in GenBank to analyze interspecific differences among the specimens (Table 2). The accession numbers of A. pegreffii (from Gymnoscopeculus nicholsi), A. simplex C (G. nicholsi), and A. simplex C (Electrona carlsbergi) are GQ131688, GQ131689, and GQ167200, respectively.

Our sequences of Anisakis simplex C and A. pegreffii were compared to selected sequences in GenBank (Table 2) to create a distance table using Dnadist (PHYLYP Version 3.67, Kimura-2-parameter; Appendix 1). A sequence of the rDNA ITS-1, 5.8S, and ITS-2 of Hysterothylacium aduncum from eelpout Zoarces viviparus, previously deposited in GenBank (AJ937673), was included as an outgroup to root the Anisakis phylogenetic tree. The maximum likelihood tree was built using Seqboot (bootstrap, 1000 replicates), Phyml (Guindon & Gascuel 2003), and Consense (PHYLYP Version 3.67). The optimal model for maximum likelihood was assessed using the Akaiake Information Criterion (AIC) as implemented in the online tool Findmodel (Leitner et al. 2005). This analysis supported the HKY plus Gamma model (Hasegawa et al. 1985) as the best fit substitution model for the data. We ran maximum likelihood using 4 gamma categories.

RESULTS

Fish stomach contents

The 75 specimens of Gymnoscopeculus nicholsi had a mean SL of 14.8 cm (range 13.2 to 15.9 cm) and a mean TW of 29.8 g (range 20.9 to 39.5 g). The prey items were of pelagic origin or associated with the benthioplagic (diurnal vertical migrations) environment. All of the identifiable prey items were small crustaceans and fish, mainly in an advanced stage of digestion. The stomachs contained calanoid copepods (F = 72.5%), Euphausia superba (F = 3.9%), Euphausia spp. (F = 54.9%), ostracods (F = 9.8%), and undetermined crustaceans (F = 11.8%) and teleosts (F = 9.8%). Sorted by quantity and weight percentage, Euphausia spp. (N = 4.3%, W = 27.3%) were followed by calanoid copepods (N = 54.7%, W = 69.6%) and E. superba (N = 4.3%, W = 27.3%). The IRI was highest for Euphausia spp. (5440.1), calanoid copepods (4110.7), and E. superba (123.2; Table 1).

Both Electrona species were smaller, with a mean SL of 8.5 cm (range 6.8 to 10.1 cm) for E. antarctica and 7.7 cm (range 6.1 to 9.3 cm) for E. carlsbergi, with a mean TW of 8.5 g (range 3.8 to 17.0 g) and 5.9 g (range 4.3 to 7.9 g), respectively. Most prey items of both fish
were pelagic crustaceans, mainly calanoid copepods and the euphausiid *Euphausia superba*. Ostracods, gammarids, and gastropods were minor components. *E. superba* was numerically predominant (N = 47.8 and 36.3% for *E. antarctica* and *E. carlsbergi*, respectively, followed by *Euphausia* spp. and calanoid copepods with values of 30.5 and 17.4% as well as 18.6 and 42.1%, respectively. The frequencies of occurrence (F%) of *E. superba* in both fish species were similar: 55.6 and 62.5%, respectively. Values of W (%) for *E. superba* and for undetermined Gastropoda for *E. antarctica* were 92.6 and 5.2%, with values of 85.7% for *E. superba* and 11.9% for *Euphausia* spp. in *E. carlsbergi* stomachs. For both fish species, the IRI was highest for *E. superba*, followed by *Euphausia* spp. and calanoid copepods (Table 1).

**Genetic identification**

The ITS-1, 5.8S, and ITS-2 sequences were determined for 25 *Anisakis* nematodes that were isolated from the 20 infected *Gymnoscopelus nicholsi* and from the 5 infected *Electrona carlsbergi*. The sequence analyses of the nematodes from the South Shetland Islands revealed 2 sibling species, *Anisakis simplex* C (n = 17) and *A. pegreffii* (n = 3). In *E. carlsbergi* only *A. simplex* C (n = 5) was identified. The lengths of the PCR product including the 3 regions ITS-1, 5.8S, and ITS-2 with flanking sequences ranged from 895 to 959 bp for *A. simplex* C and 919 to 959 bp for *A. pegreffii*. The length of the ITS-1 and ITS-2 sequences of *A. simplex* C ranged from 376 to 392 and 300 to 314 bp. The 5.8S sequences were all 157 bp long. The G + C contents were 45.5–47.2% (ITS-1), 51.0–52.3% (5.8S), and 42.4–43.9% (ITS-2). The length of the ITS-1 and ITS-2 sequences of *A. pegreffii* were 390–391 and 305–311 bp. The length of the 5.8S sequence of all samples was 157 bp. The G + C contents were 46.7–47.1% (ITS-1), 51.6% (5.8S), and 42.0–42.6% (ITS-2).

The sequence of *Anisakis simplex* C from *Gymnoscopelus nicholsi* (GQ131689) was identical to a sequence from Canada (AY26722), while the sequence of *A. simplex* C from *Electrona carlsbergi* (GQ167200) corresponded closely (99.0%) with a sequence from the coast of California (AY821736). Both sequences differed in only 2 positions. An alignment of the *A. simplex* C sequences from *G. nicholsi* and *E. carlsbergi* differed in 2 positions, 1 in the ITS-1 and 1 in the ITS-2 region. *A. pegreffii* from *G. nicholsi* (GQ131688) was identical (100.0%) to the sequence EU933997 of *A. pegreffii* from China (Fig. 2).

**Anisakis spp. composition**

*Gymnoscopelus nicholsi* was parasitized by 2 different anisakid nematodes, viz. third-stage larvae (L3) of *A. simplex* C and *A. pegreffii* (molecular identification above). Both species are members of the *A. simplex* complex (Fig. 3). They were isolated from the organs of the body cavity with a prevalence of 22.7% and a mean intensity of 1.4 for *A. simplex* C and 4.0% and a mean intensity of 1.0 for *A. pegreffii*. The anisakid parasite fauna of both analyzed *Electrona* species was different. The nematode species *A. simplex* C was recorded and genetically identified only for *E. carlsbergi* with a prevalence of 9.1%, while *E. antarctica* were *Anisakis* free. The third-stage larvae were encapsulated on the organs of the body cavity of the former (Table 1).

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**Fig. 2.** *Anisakis* spp. Consensus phylogenetic tree resulting from maximum likelihood analysis for ITS-1/5.8S/ITS-2 regions of 25 sequences. Probabilities (%) are given for internal branches. Specimen codes are listed in Table 2. Bootstrap values were calculated over 1000 replicates.
DISCUSSION

Here we have, for the first time, genetically identified 2 *Anisakis* sibling species, *A. simplex* C and *A. pegreffii*, from 2 myctophids in the high Antarctic around the South Shetland Islands. In contrast to earlier studies (e.g. Palm et al. 2007) from the same region, both myctophids are the first teleosts that have been infected with *Anisakis* species at such low latitudes.

According to Rocka (2006), adult specimens of the genus *Anisakis* (*A. similis* and *A. physeteris*) were reported from Antarctic marine mammals in the 1940s and 1950s (Johnston & Mawson 1945, Mozgovoy 1953). Dailey & Vogelbein (1991) identified *Anisakis* species from 3 migrating whales, while Mattiucci & Nascetti (2007) reported *A. simplex* C from elephant seals *Mirounga leonina*. However, no other adult *Anisakis* have been reported from the region, and their abundance in final and intermediate hosts has not been explored. Within a series of parasitological studies of the most frequent teleosts around the South Shetland Islands and in the Weddell Sea, Palm et al. (1998: *Notothenia coriiceps*; 2007: *Chaenocephalus aceratus*, *Lepidonotothen squamifrons*, *Trematomus eulepes*) could not identify any *Anisakis* larvae in the studied fish, and even the Antarctic herring *Pleuragramma antarcticum* as the predominant nototheniid species in the pelagic realm was uninfected (e.g. Bartsch 1985). This contrasts the report by Rokicki et al. (2009) on the infection with morphologically identified *Anisakis* sp.
of 11 different fish species, including *Electrona antarctica*, *L. squamifrons* (= *L. kempi*), *N. coriceps*, and *T. eulepidotus*. In the present study 2 myctophids, *Gymnoscopelus nicholsi* and *E. carlsbergi*, were found to be infected, whereas the endemic *E. antarctica* was free of *Anisakis*.

Among the studied myctophids, *Electrona antarctica* lives circumpolar south of the Antarctic convergence, while *E. carlsbergi* and *Gymnoscopelus nicholsi* also reach high biomass in more northern waters up to the Patagonian shelf (e.g. Linkowski 1985, Gon & Heemstra 1990, Brickle et al. 2009). The latter two species usually migrate into high Antarctic waters during extensive annual migrations. Both migratory myctophids were infected with *Anisakis* spp., while the most common Antarctic endemic myctophid as well as other abundant fish species (see above) were *Anisakis*-free in the area of investigation. In contrast to North Atlantic regions such as the Greenland Sea and the northern Mid-Atlantic Ridge (e.g. Klimpel et al. 2006, 2007, 2008a, Kellermanns et al. 2007), the abundance and distribution of whaleworms within different Antarctic teleosts are fairly low, especially compared to the other anisakid nematodes *Pseudoterranova decipiens* E and *Contracaecum* spp. (Klöser et al. 1992, Palm 1999). Both genera typically infect seals, whereas *Anisakis* is most common in cetaceans and only occasionally infects seals (e.g. Klöser et al. 1992, Palm 1999, Klimpel et al. 2004, 2008b). Crabeater seals *Lobodon carcinophagus*, Weddell seals *Leptonychotes weddelli*, and leopard seals *Hydrurga leptonyx* are most frequently distributed in high Antarctic waters and are the main abundant top predators (Nordøy et al. 1995, Flores et al. 2004). The elephant seal, another abundant pin-niped species, has a circumpolar distribution, and its breeding colonies are mainly concentrated on and around the sub-Antarctic islands (Slade et al. 1998).

Summarizing the available studies on fish parasites in Antarctic waters, *Anisakis* spp. seem to be rare visitors compared to other anisakid genera. This is astonishing, since cetaceans are common in high Antarctic feeding grounds, enabling the transfer of parasites within these waters. For example, minke *Balaenoptera bonaerensis* and fin whales *B. physalus*, long-finned pilot whales *Globicephala melas*, Cuvier’s beaked whales *Ziphius cavirostris*, and sperm whales *Physeter macrocephalus* occur in great numbers and biomass in sub-Antarctic and Antarctic areas north of the Antarctic peninsula and near the ice edge (e.g. Kasamatsu 2000, Williams et al. 2006). Dailey & Vogelbein (1991) reported adult *Anisakis* in sei *B. borealis*, northern minke *B. acutostrata*, and sperm whales in Antarctic commercial whaling sectors, although according to the given position data, only a single record was at 70° south in high Antarctic waters. One would there-fore expect the introduction of *Anisakis* into Antarctic waters to occur most commonly via migrating whales and one would expect a similar high *Anisakis* infection rate compared to other anisakids. However, sealworms of the genera *Contracaecum* and *Pseudoterranova* clearly dominate the Antarctic anisakid nematode fauna.

Both recorded *Anisakis* species within the present study are known for their wide distribution outside the Southern Ocean (Mattiucci & Nascetti 2008). Since its first identification by Mattiucci et al. (1997), *A. simplex* C has exhibited a scattered zoogeographical distribution, including the Canadian and Chilean Pacific coasts, waters around New Zealand, and Atlantic waters off South Africa (Mattiucci & Nascetti 2008). Adults of this species have been genetically identified from cetaceans of the families Delphinidae and Ziphiidae (e.g. Klimpel et al. 2008b, Mattiucci & Nascetti 2008; Fig. 3), and a record exists from elephant seals from the sub-Antarctic (Mattiucci & Nascetti 2008). The ITS1-2 region of our specimens from the South Shetland Islands was identical to that of specimens from Canada or California. *A. pegreffii* utilizes toothed whales of the family Delphinidae as its final hosts; however, it also infects the families Ziphiidae, Physeteridae, and Neobalaenidae (Mattiucci et al. 1997; Fig. 3), mainly in the Atlantic Ocean and the Mediterranean Sea but also in Australian waters. The specimens from *Gymnoscopelus nicholsi* were genetically identical to specimens from China. However, both species are at their most southern distribution in the Southern Ocean.

Here we identified migrating myctophids (distributed in- and outside the Southern Ocean) as common teleost intermediate hosts for the whaleworms *Anisakis* spp. in sub-Antarctic waters. In contrast, the endemic *Electrona antarctica* as well as all benthode-mersal and pelagic fish species that we studied were *Anisakis* free. It appears that independent of earlier records of *Anisakis* in Antarctica (see above), our specimens may have migrated into these waters through their myctophid hosts. *A. simplex* C is common along the South American coast, and *A. pegreffii* is very common in mid-Atlantic waters. This would suggest that the record of *A. simplex* C by Mattiucci & Nascetti (2008) represents an accidental case of infection in elephant seals. It seems that the Antarctic waters are at the southern range for both recorded *Anisakis* species. The studied myctophids had only few prey items within their stomachs, and these were clearly dominated by euphausiids including *Euphausia superba* and planktonic copepods. Especially *E. superba* plays an important role as a key prey item of numerous top predators and is the most common prey for pelagic myctophids in Antarctic waters (Pusch et
al. 2004). Euphausiids and copepods are known as typical first intermediate hosts, while pelagic fish species are second or paratenic hosts in the life cycle of Anisakis species outside the Southern Ocean (e.g. Klimpel et al. 2004, 2008b). Regulation of the nematode populations off the South Shetland Islands must then be expected to take place in the first crustacean intermediate host, because a survey of more than 50,000 E. superba revealed no nematode larvae (Kagei et al. 1978). If nematode populations were strictly controlled in the first intermediate host, the numbers of parasites might be too low to allow significant transmission into the fish intermediate hosts in Antarctic waters. Furthermore, Delphinidae, the typical final hosts in the life cycles of A. simplex and A. pegreffii (Fig. 3) are missing in the Southern Ocean, while only some of the secondary (Fig. 3) final hosts (e.g. migrating whales of the Physeteridae and Ziphiidae) are known to occur in the Antarctic. This can explain the lower abundance of Anisakis spp. in high Antarctic waters.

CONCLUSIONS

Our study confirms that 2 Anisakis species, A. simplex and A. pegreffii, occur in Antarctic waters. Our specimens were genetically identical to specimens recorded earlier from Pacific Canada (A. simplex C) and from China (A. pegreffii), demonstrating the extensive range of their distribution. Comparison to sequences from GenBank identified this as a common pattern in other Anisakis species as well, which have a wide distribution with minor genetic changes of the ITS1-2 regions. The occurrence in migrating myctophids with rare findings from other teleosts leads to the conclusion that both Anisakis species originated outside the Antarctic in more northern waters, where delphinids serve as common final hosts. The presence of phystoderids and ziphhiids in Antarctic waters and the near absence of Anisakis in most endemic Antarctic fish suggests that these whelworms are transported into the Antarctic through their migrating teleost intermediate hosts. Delphinids have been identified as the main final hosts for A. simplex and A. pegreffii (e.g. Klimpel et al. 2008b, Mattiucci & Nascetti 2008), while other cetaceans and elephant seals serve as secondary (additional) or erroneous hosts, respectively. Regular introduction events have likely led to the infection by Anisakis of Antarctic fish, bringing into question the existence of stable Anisakis populations. Further studies and the search for Anisakis spp. in Antarctic waters are needed to confirm these preliminary interpretations.

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LITERATURE CITED


Kellermanns E, Klimpel S, Palm HW (2007) Molecular identification of ascaridoid nematodes from the deep-sea onion-


Linkowski TB (1895) Population biology of the myctophid fish Gymnoscelus nicholsi (Gillbert, 1911) from the western South Atlantic. J Fish Biol 27:683–698


Mozgovoy AA (1953) Ascaridata of animals and man, and the diseases caused by them. Osnovy nematologii II. Izdatelstvo AN SSSR, Moskva (in Russian)


Pinkas L, Oliphant MS, Iverson ILK (1991) Food habits of albacre, bluefin tuna, and bonito in Californian waters. Calif Fish Game Fish Bull 152


Zdzitowiecki K (1991) Occurrence of digeneans in open sea fishes off the South Shetland Islands and South Georgia, and a list of fish digeneans in the Antarctic. Pol Polar Res 12:55–72


Zdzitowiecki K, Laskowski Z (2004) Helminths of an Antarctic fish, Nototthenia coriceps, from the Vernadsky Station (Western Antarctica) in comparison with Admiralty Bay (South Shetland Islands). Helminthologica 41:201–207


Appendix 1. Computed distance-matrix under Kimura-2-parameter substitution model among the 25 sequences of *Anisakis* spp. (24) and *Hysterothylacium aduncum* (1) for ITS-1/5.8S/ITS-2 sequences. Transition/transversion ratio = 2.0. Abbreviations as in Table 2

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