



Report on anisakid nematodes in polar regions – Preliminary results

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Abstract

The aim of this study is to extend our knowledge of the distribution of anisakid nematode parasites in Arctic and Antarctic polar regions. We examined vertebrate (fish) taxa characteristic of the faunas in both polar regions for the presence of parasitic nematodes. The material was collected from Svalbard (Arctic) between July and August 2008 and from King George Island (South Shetland Islands, Antarctic Peninsula) between November 2007 and January 2008. In addition, faecal, bird, and invertebrate samples were collected and examined for the presence of anisakid nematodes or eggs. *Anisakis simplex* s.s. was found in the body cavity of Arctic cod, and *Contracaecum* sp. and *Pseudoterranova* sp. were found in Antarctic notothenioids. Eggs of *Anisakis* sp. and *Contracaecum* sp. were recovered from the faeces of *Mirounga leonina*. We present the first record of the occurrence of *A. simplex* C in the Antarctic fishes *Notothenia coriiceps* and *Notothenia rossii*.

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1. Introduction

The polar regions include the land and water located north of 60°N (Arctic) and south of 60°S (Antarctic). There are no land mammals in the Antarctic, where the main inhabitants are marine mammals (whales, porpoises, seals) and birds. In contrast, the Arctic is home to terrestrial mammals such as reindeer, fox, wolf, lemming, caribou, musk ox, hare, and polar bear.

Members of the family Anisakidae (Nematoda: Secernentea) include parasitic species that occur in both the Northern and Southern Hemispheres, and that are of veterinary, medical, and economic importance. Some species are also known to be dangerous to humans (van Thiel, 1962). Adults of most of these parasites live in the alimentary tract of the vertebrate host (mammals, birds, fish), and are present in both marine and freshwater environments. The life cycles of numerous taxa involve the development of larval stages in one or more intermediate hosts (fish, invertebrates). The differences between southern and northern trophic webs may affect the distribution of parasites in these two regions.

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The aim of this study and short report is to extend knowledge of the distribution of anisakid parasites in both polar regions.

2. Materials and methods

The study material was collected on King George Island (South Shetland Islands, Antarctic Peninsula) and on Svalbard (Table 1).

2.1. Arctic material

The Arctic study material was collected close to the Polish Polar Station at Hornsund on Svalbard during the summer of 2008 (13 July to 20 August).

Fish samples, including *Liparis liparis* (Montagu's sea snail), *Salvelinus alpinus* (Arctic char), *Myoxocephalus scorpius* (short-spined sea scorpion), and *Boreogadus saida* (polar cod, Arctic cod), were collected in the Hornsund Fjord area, mainly from Isbjornhamna and Ariebukta. Further samples of Arctic char were collected from two freshwater lakes,

Revvatnet and Sartvatnet (Sorkapp Land), and from the Revelva River. All the fish were examined for the presence of anisakid nematodes. Nematodes were fixed in either 70% ethanol or 70% ethanol with 5% glycerol.

We also collected vertebrate faecal samples, including that of *Ursus maritimus* (polar bear), *Rissa tridactyla* (black-legged kittiwake), *Sterna paradisaea* (Arctic tern), *Uria lomvia* (Brünnich's guillemot), and *Branta leucopsis* (barnacle goose). All samples were obtained from the Ariedalen Valley and Fuglebergsletta, except for those of polar bear, which were collected near Gnalberget. Two methods were used for parasite egg recovery: flotation and decanting. In the flotation method, the faecal material was strained through a sieve (1 mm mesh) into a Petri dish containing a salt solution. After 15–20 min, the liquid was transferred to centrifuge tubes and a coverslip was placed in contact with the meniscus. The coverslip was then placed on a slide, and examined for parasite eggs under the microscope (5–10× magnification). For decanting, the collected material was placed in water,

Table 1
Anisakid nematode eggs/nematodes found in samples investigated in the present study.

Region	Sample type	Host species	Anisakids found/no. of samples examined	Parasite taxa ^b (location within host body ^a)
Arctic (Svalbard)	Faecal	<i>Ursus maritimus</i>	0/7	
		<i>Rangifer tarandus playrhynchus</i>	0/4	
		<i>Rissa tridactyla</i>	0/3	
		<i>Sterna paradisaea</i> , <i>Uria lomvia</i>	0/4	
		<i>Branta leucopsis</i>	0/6	
		<i>Liparis liparis</i>	0/3	
	Fish	<i>Salvelinus alpinus</i>	0/40	
		<i>Myoxocephalus scorpius</i>	0/2	
		<i>Boreogadus saida</i>	1/2	<i>A. sim.</i> (bc)
	Invertebrate	<i>Gammarus oceanicus</i>	0/59	
	Bird	<i>Larus hyperboreus</i>	0/1	
<i>Alle alle</i>		0/1		
Antarctic (King George Island)	Faecal	<i>Mirounga leonina</i>	9/9	<i>A. sp.</i> (f); <i>C. sp.</i> (f)
		<i>Pygoscelis adeliae</i>	0/5	
		<i>Pygoscelis papua</i>	0/5	
		<i>Pygoscelis antarcticus</i>	0/6	
		<i>Stercorarius antarctica</i>	0/2	
		<i>Macronectes giganteus</i>	0/1	
		<i>Chaenocephalus aceratus</i>	10/10	<i>P. sp.</i> (bc, l, s)
	Fish	<i>Notothenia rossii</i>	8/21	<i>A. sim.</i> C (bc); <i>P. sp.</i> (bc, l)
		<i>Notothenia coriiceps</i>	34/61	<i>A. sim.</i> C (bc); <i>P. sp.</i> (bc, l); <i>C. sp.</i> (bc)
		<i>Harpagifer antarcticus</i>	0/2	
		<i>Lepidonotothen nudifrons</i>	0/7	
		<i>Trematomus newnesi</i>	0/1	
		<i>Trematomus bernacchii</i>	0/1	

^a bc: body cavity; l: liver; s: stomach; f: faeces.

^b *A. sim.*: *Anisakis simplex* s.s.; *A. sp.*: *Anisakis* species; *A. sim. C*: *Anisakis simplex* C; *C. sp.*: *Contraecum* species; *P. sp.*: *Pseudoterranova* species.

and the supernatant was poured off after 30, 50, and 70 min; the sediments were then examined under a microscope.

Fifty-nine individuals of the crustacean *Gammarus oceanicus* were examined for the presence of parasitic nematodes; we also examined dead specimens of two bird species: *Larus hyperboreus* (glaucous gull) and *Alle alle* (little auk).

2.2. Antarctic material

The Antarctic material was collected between November 2007 and January 2008 near Arctowski Station on King George Island, where fish were caught in Admiralty Bay.

Seven fish species were examined: *Chaenocephalus aceratus* (blackfin icefish), *Notothenia rossii* (marbled rockcod), *Notothenia coriiceps* (black rockcod), *Harpagifer antarcticus* (Antarctic spiny plunderfish), *Lepidonotothen nudifrons* (gaudy notothen), *Trematomus newnesi* (dusky notothen), and *Trematomus bernacchii* (emerald rockcod). Vertebrate faecal samples were obtained from *Mirounga leonina* (southern elephant seal), *Pygoscelis adeliae* (Adélie penguin), *Pygoscelis papua* (gentoo penguin), *Pygoscelis antarcticus* (chinstrap penguin), *Stercorarius antarctica* (brown skua), and *Macronectes giganteus* (southern giant petrel). Each faecal sample contained between 10 and 15 stools, and was examined as described above.

2.3. Nematode identification

Parasitic nematodes and their eggs were identified based on morphological characteristics (Hartwich, 1974; Stefański et al., 1952). In addition, PCR-RFLP (Restriction Fragment Length Polymorphism) analysis of the nuclear rDNA region containing Internal Transcribed Spacers and the 5.8S rDNA gene were used to aid species identification of *Anisakis* spp. larvae from fish (D'Amelio et al., 2000; Pontes et al., 2005), following the method of Kijewska et al. (2009). PCR products were digested with three restriction endonucleases (*TaqI*, *HinfI*, *HhaI*; Fermentas, Lithuania). Restriction with *TaqI* and *HinfI* produced characteristic bands representing three fragments (430, 400, and 100 bp) and two fragments (620 and 250 bp), respectively, for both *Anisakis simplex* s.s. and *A. simplex* C. Restriction with *HhaI* produced two fragments for *A. simplex* s.s. (550 and 430 bp) and three fragments for *A. simplex* C (550, 430, and 130 bp). Previous studies have shown that the use of these three enzymes enables the differentiation of all *Anisakis* species that

cannot be discriminated based on morphological characteristics alone (D'Amelio et al., 2000; Pontes et al., 2005).

3. Results

Parasitic nematodes were recovered from *N. coriiceps*, *N. rossii*, *C. aceratus*, and *B. saida*. Preliminary morphological analyses revealed the presence of *Anisakis* sp., *Contracaecum* sp., and *Pseudoterranova* sp. *Contracaecum* sp. was found in the body cavity of *N. coriiceps*. *Pseudoterranova* sp. was found in the body cavity and liver of *N. coriiceps*, *N. rossii*, and *C. aceratus*. A single anisakid nematode (*A. simplex* s.s.) was found in the body cavity of *B. saida*. Individuals of *A. simplex* C were found in the body cavity of *N. coriiceps* and *N. rossii*.

Parasite eggs were found only in faecal samples of *M. leonina*. A total of 70 eggs were found, and morphological examination confirmed the presence of *Anisakis* sp. (95% of eggs) and *Contracaecum* sp. (5% of eggs). Future work to confirm species identities will be based on the application of molecular techniques.

In the present study, *A. simplex* C was obtained for the first time from *N. coriiceps* and *N. rossii*. Previously, it has been reported from Delphinidae, Gemnyliidae, Moridae, and Pinguipedidae (from the South African coast, North-East Pacific, New Zealand, and Tasman Sea), and occasionally from *M. leonina* (sub-Antarctic area) and *Mirounga angustirostris* (North-East Pacific) (Mattiucci et al., 1997; Mattiucci and Nascetti, 2006, 2008; Nadler et al., 2000). The present study extends the range of occurrence of this species to include Admiralty Bay, South Shetland Islands (maritime Antarctic).

4. Discussion

The marine fauna of the Antarctic is highly endemic (Rogers, 2007), comprising many species that do not occur elsewhere, and excluding many familiar species from lower-latitude faunas. This generalization applies to both host and parasite species. In the Antarctic, six species of anisakid nematodes have been reported to date, of which five are currently thought to be endemic: *Contracaecum radiatum*, *Contracaecum osculatum* D and E, *Pseudoterranova decipiens* E, and *Contracaecum miroungae* (Bullini et al., 1997; Kloser et al., 1992; Nadler et al., 2000; Orecchia et al., 1994; Palm, 1999). In the Arctic, *C. osculatum* A (Nascetti et al., 1993) and *Pseudoterranova bulbosa* (Paggi et al., 1991) have been reported, and several other species

occur in sub-Arctic areas (Mattiucci and Nascetti, 2008). These parasites are characterized by their specificity, being limited to a small number of final hosts, and, indirectly, a life cycle with specific Antarctic or Arctic intermediate hosts. Anisakid nematodes have a complex life cycle involving several host species. Depending on the species, the definitive hosts of these nematodes include marine mammals, birds, and fish. The life cycle also involves one or more intermediate or paratenic hosts such as invertebrates (euphausiids, squid) and fish. Other species, such as the polar bear, can potentially become infected by anisakid nematodes by eating infected seal or fish. Crustaceans form part of the diets of the fish and seal species identified in this study to be infected with anisakids (Iken et al., 1997; McKenna, 1991). Euphausiids (krill) and squid are known to be intermediate hosts of marine nematode parasites worldwide. Little is known of the infection of invertebrates by anisakid nematodes in the Antarctic; consequently, our knowledge of transmission mechanisms remains limited.

The Antarctic fish examined in this study belong to three families: Harpagiferidae (*H. antarcticus*), Chanichthyidae (*C. aceratus*), and Nototheniidae (all other species). All belong to the suborder Notothenioidei, which dominates the Antarctic shelf and is characteristic of this region (Eastman, 2005). Due to their dominance, these species are considered as the most likely intermediate hosts for anisakids. This hypothesis was supported by the data obtained in the current study, and most of the anisakids were found in the above species. In turn, the Arctic fishes (Gadidae (*B. saida*), Cottidae (*M. scorpius*), Liparidae (*L. liparis*), and Salmonidae (*S. alpinus*)) were classified as typical intermediate hosts for Anisakidae species. These species are also commonly found in temperate waters of the Northern Hemisphere; however, in this study we found only a single specimen of *A. simplex* s.s. in the body cavity of a *B. saida* from Hornsund Fjord. This specimen was a juvenile and its size suggested that this fish originated from shoal feeding on offshore grounds inside the fjord, possibly explaining the poor parasitic fauna found during the examination. The distribution of eggs and larvae in animals other than fishes revealed the presence of Anisakidae in the faecal matter of *M. leonina*, whereas birds and terrestrial mammals, including polar bears, appeared to be free from anisakids.

The lack of seals and whales or dolphins in the northern sample introduces some uncertainty into our conclusions. However, available data indicate a high probability that the most important group in terms of anisakid distribution in polar regions is fish-eating

mammals, as also concluded by Mattiucci and Nascetti (2008), although the presence of fish within the diet of potential final hosts does not necessarily mean that these species are able to serve as final hosts. Examination of the faeces of various fish-eating birds and mammals (polar bear), and examinations of the bodies of some birds (Table 1), revealed that these species are not anisakid hosts.

Anisakid nematodes occur worldwide, including the Arctic and Antarctic regions; however, while these two regions appear to be similar in terms of environmental conditions, they differ with respect to the dominant hosts and parasite taxa. This discrepancy possibly reflects species divergence and the narrow specificity of parasites living in only one or several hosts, as with *C. radiatum* and *Anisakis physeteris* (Mattiucci and Nascetti, 2008), in contrast to the worldwide distribution of wide-specificity parasites such as *A. simplex* s.s. and *Anisakis pegreffii*. The known phylogenies of Anisakidae, constructed based on various molecular genetic datasets (Kijewska et al., 2009; Nadler et al., 2000; Zhu et al., 2002), reveal that the degree of parasite specificity may be strongly related to the geographical distribution of the host. This factor determines the distribution of parasites and affects the number and choice of intermediate host species, although there exists a lack of data regarding the distribution of some species (for example *A. simplex* C, reported here for the first time in *N. coriiceps* and *N. rossii*).

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