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Parasites of the Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937) (Perciformes, Nototheniidae) in the Pacific sector of the Antarctic

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Abstract

The Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937) is one of the main target species of commercial fisheries in the Antarctic. It is an endemic and is found along the shelf of Antarctica, as well as on the slopes of seamounts, underwater elevations and islands in the sub-Antarctic. It feeds on a variety of fish and cephalopods and can be an intermediate/paratenic host of some helminthes, whose final hosts are whales, seals, large rays and sharks. This article presents new data on toothfish infection in the Pacific sector of the Antarctic. Specimens were examined during commercial longline fishing in the Ross Sea and the Amundsen Sea in January–February 2013. Fourteen species of parasites were found using standard parasitological methods and genetic analysis.

The Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937) is one of two species in the genus and one of the main target species of commercial fisheries in the Antarctic. *Dissostichus mawsoni* is endemic, with a circumpolar distribution along the shelf of Antarctica and on the slopes of seamounts, underwater elevations and islands in the sub-Antarctic (Shust & Petrov 2009). The history of targeted research on the Antarctic toothfish and subsequent exploratory fishing began in 1969 (Calhaem & Christoffel 1969), but its life cycle and biology are still poorly understood because its habitat is covered by sea ice from March to December (Juhov 1982; Andrijašev 1986; DeWitt in Gon & Heemstra 1990; Shust et al. 2005; Hanchet 2006).

The study of Antarctic toothfish parasites includes a few studies carried out mainly in the Atlantic sector of the Southern Ocean. Sixteen species of helminthes were found near the Antarctic Peninsula (e.g., Rodjuk 1984; Gaevskaja et al. 1990; Zdzitowiecki 1990, 1991a; Rocka 2003, 2004; Rokicki et al. 2009). Three species of trematodes were found in the Ross Sea (Holloway & Bier 1968; Sokolov & Gordeev 2013, 2015) as well as the copepod *Eubrachiella antarctica* Quidor, 1906 (Smith et al. 2006). Two species of Monogenea were recorded in the Pacific sector of the sub-Antarctic (Gaevskaja et al. 1990) and in the Antarctic (Oguz et al. 2012).

Material and methods

A total of 20 adult specimens of *Dissostichus mawsoni* (total length 97–159 cm; weight 11.23–55.04 kg) were caught between 21 and 27 January 2013 in the central part of the Ross Sea. Five specimens were caught on 3 February 2013 in the Amundsen Sea from the fishing vessel *Yantar-35* when fishing for toothfish at depths ranging from 865 to 1425 m using Mustad Autoline bottom longline fishing gear inside the Convention for the Conservation of Marine Living Resources area. All specimens were caught on the continental slope between 75°25′ S, 174°42′ W and 69°44′ S, 126°46′ W. Straight after capture all specimens were examined macroscopically for the presence of

ectoparasites and then dissected and studied for helminthes and other metazoan parasites using a standard method of parasitological examination (Byhovskaja-Pavlovskaja 1985). Prefixation treatment of trematodes, cestodes and monogeneas included cleaning and straightening with the compression method for better identification. Acanthocephala were transferred to freshwater until the proboscis everted prior to fixation. All parasites except nematodes were preserved in 70% and 96% ethanol. Nematodes were preserved in 4% formaldehyde and three days later transferred to 70% ethanol for longterm storage. Some nematodes were preserved in 96% ethanol. Digenea were stained with acetic carmin, dehvdrated, contrasted (cleared) with dimethyl phthalate or diethyl phthalate, and finally mounted in Canada balsam. Cestoda were hydrated, stained with Harris's hematoxylin, differentiated in tap water, de-stained in ethanol, dehydrated, cleaned in methyl salicylate, and finally mounted in Canada balsam (Jensen et al. 2011).

Fish species were identified with reference to Gon & Heemstra (1990). Parasites were identified with the aid of literature, including Holloway & Bier (1968), Zdzitowiecki (1991b), Rocka & Zdzitowiecki (1998), Zdzitowiecki (1999), Rocka (2003), Smith et al. (2006) and Bray et al. (2008). Tetraphyllidean and onchoproteocephalidean larvae were identified using DNA sequencing.

DNA extraction was carried out using the standard saline method (Aljanabi & Martinez 1997). Amplifications were performed in a final reactions volume of 12.5 µl: 1.5 µl PCR buffer (Sileks, Russia); 2.5 mm MgCl₂; 0.6 mm dNTP; 2 pmol of each primer; 200 ng DNAmatrix; and 1 unit of Taq-polymerase (Sileks, Moscow) with the following cycle protocol: a preliminary denaturation of DNA at 94°C for two minutes was followed by the syntheses of DNA products: melting at 94°C for 30 s, annealing the primers at 55°C for 45 s, and DNA syntheses at 72°C for two minutes. A final extension cycle of five minutes at 72°C followed. Amplification of lsuDNA was performed using primers 1200R (5'-GCATAGTTCACCATCTTTCGG-3') (Lockyer et al. 2003) and LSU5 5'-TAGGTCGACCCGCTGAAYTTAAGCA-3') (Littlewood et al. 2000). Sequencing was carried out with BigDye version 1.1 on the automatic sequencer ABI 3100. Results processing and multiple alignment were carried out using the software package DNAStar (Lasergene Inc.).

Results

A total of 14 parasite taxa were detected (Table 1). The copepod *Eubrachiella antarctica* was found on and near the dorsal, pectoral and anal fins. The monogenean

Pseudobenedenia dissostichi Timofeeva, Gaevskaja & Kovaliova, 1987 was found mainly between the pectoral fins, but sometimes on the rest of the body surface, except the head. Other species not listed in Table 1—leeches (Hirudinea) *Moorebdellina* sp. and *Pleurobdella* sp.—were very common on *Dissostichus mawsoni*, but infection indices of these species are not available because of the lengthy contact between examined specimens in the fish-receiving.

Two morphological types of plerocercoid were found: small (1–2 mm) with bilocular bothridia, and large (2–4 mm) with trilocular bothridia. Sequences of small plerocercoids with bilocular bothridia exhibited a 95% match with *Calyptrobothrium* sp. (Genbank no. AF382087.1) collected by Brickle et al. (2001) from *Apristurus laurussonii* (Saemundsson, 1922) (formerly accepted as *Apristurus atlanticus* Koefoed, 1932) caught in the North Sea. Large plerocercoids with trilocular bothridia showed 99% match with *Onchobothrium antarcticum* Wojciechowska, 1990 (Genbank no. KF882021.1) collected by Laskowski & Rocka (2014) from *Notothenia rossii* Ricardson, 1844 which were caught around the South Shetland Islands.

Discussion

In comparison with other high-level predators from the near-continental areas of the ocean, data on the parasites of Dissostichus mawsoni are fragmentary and scarce. Only seven of the 14 species found in our study were previously recorded as parasites of D. mawsoni: Neolepidapedon trematomi Prudhoe & Bray, 1973, Helicometra antarcticae Holloway & Bier, 1968, Onchobothrium antarcticum, E. antarctica, Hysterothylacium sp., Echinorhynchus petrotschenkoi (Rodjuk 1984) and Corynosoma bullosum Linstow, 1892. Neolepidapedon trematomi and Corvnosoma bullosum Linstow, 1892 were found by Zdzitowiecki (1987 1988, 1990, 1999) in the South Shetland Islands area and the first of the above-mentioned species by Sokolov & Gordeev (2013), Sokolov & Gordeev (2015) in the Ross Sea. Helicometra antarcticae was described by Holloway & Bier (1968) from samples taken from D. mawsoni caught in McMurdo Sound (Ross Sea), and we later found specimens in the open waters of the Pacific sector and in the Ross Sea (Sokolov & Gordeev 2013, 2015). Eubrachiella antarctica on the trunks of D. mawsoni in the Ross Sea was considered to be a marker or biological tag to aid stock discrimination (Smith et al. 2006). The plerocercoid O. antarcticum and the nematode Hysterothylacium sp. were recorded living on this species in Admiralty Bay and the open sea shelf near the South Shetlands Islands (Rocka 2003, 2004, respectively). Metechinorhynchus petrotschenkoi-later accepted as

Table 1	Prevalence and	intensity of	f infection	and site of	parasites in D.	mawsoni fro	om the Ross a	nd Amundsen sea
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		Ross Sea (n = 20)		Amundsen Sea ($n = 5$)		
Parasite	Adult (a)/ larva (l)	Prevalence (%)	Mean intensity (range)	Prevalence (%)	Mean intensity (range)	Site
Copepoda						
Eubrachiella antarctica	а	60	12 (1-86)	36	3 (1-6)	Body surface
Monogenea						
Pseudobenedenia dissostichi	а	55	11 (1-36)	40	8 (8)	Body surface
Acanthocephala						
Corynosoma bullosumª	I.	5	10 (10)	_	_	Intestine, pyloric caeca
Corynosoma sp.ª	I	_	_	20	1 (1)	Intestine, pyloric caeca
Echinorhynchus petrotschenkoi	а	10	4 (2-6)	40	9 (9–10)	Intestine
Echinorhynchus sp.	а	5	2 (2)	20	1 (1)	Intestine
Nematoda						
Contracaecum sp.ª	I	5	1 (1)	_	_	Intestine
Hysterothylacium sp.	a/l	100	25 (1–90)	40	23 (32–83)	Intestine, pyloric caeca, stomach
Trematoda						
Helicometra antarcticae	а	55	33 (1-243)	20	7 (7)	Intestine, pyloric caeca
Neolepidapedon trematomi	а	50	16 (1-78)	40	113 (22-204)	Intestine, pyloric caeca
Proctophantastes gillissi	а	_	_	40	1 (1-2)	Intestine
Cestoda						
Onchobothrium antarcticum	I	15	39 (1-108)	60	10 (2-21)	Intestine, pyloric caeca
Diphyllobothriidae gen. sp.	I	15	3 (1-6)	40	1 (1)	Body cavity, stomach wall
Calyptrobothrium sp.	I	15	173 (6–471)	60	27 (11–57)	Intestine, pyloric caeca

^aAberrant parasite.

E. petrotschenkoi—was found in the South Shetland Islands area (Rodjuk 1984).

Contracoecum sp., Proctophantastes gillissi (Overstreet & Pritchard, 1977) Bray & Gibson, 1986, P. dissostichi, Diphyllobothriidae gen. sp. larvae and Calyptrobothrium sp. were recorded as parasites of *D. mawsoni* for the first time. It seems that toothfish is a postcyclic host of P. gillissi and becomes infected by feeding on Muraenolepis (Sokolov et al. 2016). Corynosoma sp. and Echinorhynchus sp. are not included in the list of parasites found for the first time because the poor condition of specimens made proper identification impossible. It should be noted that in the present study larvae of C. bullosum, Corynosoma sp. and Contracaecum sp. are most likely aberrant parasites of D. mawsoni. Since the larvae of these species occur mainly in the body cavity and musculature of fish, their localization in the intestine (Table 1) of the examined fishes is atypical.

Dissostichus mawsoni is most heavily infected by cestode larvae. Because of their distribution via planktonic copepods cestodes larvae are wide spread and are found in species of many families: Nototheniidae, Artedidraconidae, Bathydraconidae, Macrouridae, Channichthyidae, Myctophidae and Muraenolepididae (Rocka 2003). After being planktophagous during their short juvenile period, they then become predatory, feeding variously on bony fish, squid and octopus up to a depth of 2250 m (Petrov & Istomin 2010). With growth, Antarctic toothfish become one of the biggest predators in the Antarctic waters (max. > 200 cm long) and are only usually eaten by sharks and whales. Therefore, tetraphyllidean larvae and plerocercoids, representing the recently created order Onchoproteocephalidea (Caira et al. 2014), accumulate in this dead-end host, reaching significant infection rates.

Other than the parasites listed in this study other researchers have found six species of trematodes: Helicometra rakusai Zdzitowiecki, 1997 in the Ross Sea (Sokolov & Gordeev 2013), Lecithaster macrocotyle Szidat & Graefe, 1967, Macvicaria pennelli Leiper & Atkinson, 1914, Gonocerca phycidis Manter, 1925, Lepidapedon garrardi Leiper & Atkinson, 1914, Neolebouria georgiensis Gibson, 1976 in the South Shetland Islands area (Zdzitowiecki 1987, 1988, 1991a, 1999). Moreover, Zdzitowiecki found Metacanthocephalus dalmori Zdzitowiecki, 1983, Corynosoma hamanni Linstow, 1892 and Corynosoma pseudohamanni Zdzitowiecki, 1984 on D. mawsoni in the South Shetland Islands area (1990). Anisakis sp. larvae and Pseudoterranova decipiens Krabbe, 1878 larvae were found on the same host and in the same area (Rokicki et al. 2009) as well as the monogenean Neopavlovskioides georgianus Kovaljova & Gaevskaja, 1977 (Gaevskaja et al. 1990). Two more monogenean species were found on *D. mawsoni* in the Pacific sector of sub-Antarctica and near the Palmer Archipelago: *Neopavlovskioides dissostichi* Dillon & Hargis, 1968 and *Pseudobenedenia nototheniae* Johnston, 1931 (Gaevskaja et al. 1990; Oguz et al. 2012, respectively).

Infection of *Dissostichus eleginoides* Smitt, 1898, a congener whose habitat overlaps with *D. mawsoni* throughout the Antarctic, including the Ross Sea, has been studied in much more depth (Brickle et al. 2006; Brown et al. 2012). Despite similar biology and feeding behaviour (Shust et al. 2009; Collins et al. 2010), *D. mawsoni* shares only 5 of 13 parasites recorded for *D. eleginoides* in the Ross Sea: *H. antarcticae*, the tetraphyllidean plerocercoid, *Hysterothylacium* sp., *C. bullosum* and *E. petrotschenkoi* (Brickle et al. 2005). Possible explanations for this are the different size ranges of the samples collected and possibly also that specimens of *D. eleginoides* were caught close to or inside the in-shelf depressions of the Ross Sea, where the main food sources of *Dissostichus* spp. are quite different to those on slopes and in deeper water.

Antarctic toothfish have been found to have low genetic diversity and temporal stability, so that even specimens collected thousands of kilometres apart are genetically similar (Mugue et al. 2014). Additionally, most tagged and recaptured fish were recorded less than 50 km from the place of their release and only 20% of fish moved more than 50 km (Dunn et al. in Petrov & Tatarnikov 2010). Although they form the foundation of an important baseline, our data are limited, and it is therefore difficult to make assumptions about the parasitofauna of D. mawsoni around the Antarctic, their biogeography, or indeed their potential as indicators of host biology. However, in their study of the parasites of D. eleginoides caught around Antarctica, Brickle et al. (2005) discovered that specimens found near Heard Island, Macquarie Island and the Prince Edward Islands were the most similar, while those from the Ross Sea was the most dissimilar, based on Sørensen's Similarity Index.

Despite the fact that Antarctic toothfish is an endemic to the Antarctic, its parasitofauna includes both cosmopolitan as well as the local species found in its congener and other bony fishes.

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