



## Spatial distribution, host specificity and genetic diversity of *Onchobothrium antarcticum* in the Southern Ocean

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**Abstract:** In this work we summarize the current knowledge on the spatial distribution, host specificity and genetic diversity of *Onchobothrium antarcticum*, an endemic Antarctic cestode. We recorded it in seven fish species, elasmobranchs *Amblyraja georgiana*, *Bathyraja eatonii*, and *B. maccaini* and teleosts *Antimora rostrata*, *Chionobathyscus dewitti*, *Dissostichus mawsoni*, and *Muraenolepis marmorata*, caught in the Ross Sea, the D'Urville Sea, the Mawson Sea, and the Weddell Sea. The infection of *A. rostrata* from the part of its distribution to the south of the Falkland Islands is reported for the first time. We obtained partial 28S rDNA and cox1 sequences of plerocercoids and adults of *O. antarcticum* and analyzed them together with a few previously published sequences. Based on the results of the phylogenetic analysis, we cannot rule out that *O. antarcticum* is in fact a complex of cryptic species.

Keywords: Antarctic, parasite, cestodes, rDNA, polar.



## Introduction

The Southern Ocean is one of the most understudied marine areas. Parasitological research of Antarctic deep-water fish is a particularly challenging task due to the difficulty in obtaining research material. An important source of scientific information on this topic is the Scheme of International Scientific Observation (SISO) of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), which provides opportunities for the scientists to collect samples from deep-water fish: the main fishery targets the toothfish *Dissostichus* spp. and by-catch such as channichthyids, macrourids, and morids (Gordeev and Sokolov 2016).

There are few genetic data on parasites from the Antarctic. An integrated approach involving genetic and morphological methods is the best tool for studying their biodiversity. Using this approach, it is possible to revisit the taxonomic position of cryptic parasitic species, develop phylogenies and obtain new data on species divergence. Most of the parasitological studies in the Antarctic have been made in shallow waters near the northern tip of the Antarctic Peninsula (Zdzitowiecki *et al.* 1997; Oğuz *et al.* 2015; Muñoz and Cartes 2020). At present, 21 species of cestodes from seven orders are known from the Antarctic fish (Rocka 2017; Polyakova and Gordeev 2020). There are 15 species of elasmobranchs (five sharks and ten rays) within the Antarctic Convergence Zone (Duhamel *et al.* 2014; Froese and Pauly 2021). Five of them, four rajids and one shark, are recorded as hosts of twelve cestode species (Wojciechowska 1990a,b; 1991a,b; Wojciechowska *et al.* 1995; Rocka and Zdzitowiecki 1998; Ivanov and Campbell 2002; Rocka 2003, 2017; Laskowski and Rocka 2014; Polyakova and Gordeev 2020). There are almost no data on the genetic diversity of Antarctic cestodes. Only two species, *Onchobothrium antarcticum* Wojciechowska, 1990 collected in the Brasfield Strait (Laskowski and Rocka 2014) and *Calyptrorhynchium* sp. from the Ross Sea and the Amundsen Sea (Gordeev and Sokolov 2016), have been examined in this aspect. Host specificity and spatial distribution of *O. antarcticum* are poorly known. There are only four records of the hosts: *Bathyraja maccaini* Springer, 1971 in the Weddell Sea (Rocka and Zdzitowiecki 1998), *Bathyraja eatonii* (Günther, 1876), *Notothenia rossii* Richardson, 1844 (Laskowski and Rocka 2014), and Antarctic toothfish *Dissostichus mawsoni* Norman, 1937 (Gordeev and Sokolov 2016).

The aim of this study was to report new hosts and localities of *O. antarcticum* in the Antarctic and to provide new data on its genetic diversity (28S rDNA and cox1).

## Material and methods

Fish specimens for the parasitological study were caught during commercial fishing for the toothfish, *Dissostichus* spp., in the CCAMLR area of responsibility using bottom longline of various constructions (Petrov *et al.* 2014) in 2010-2011 from FV *Insung 7* (INSUNG Corp.), in 2011-2012 from FV *Yantar-31* (ORION Ltd.), and in 2012-2013 and 2014-2015 from FV *Yantar-35* (ORION Ltd.). Most specimens were caught in the Ross Sea, where the main fishing grounds are located, while others were caught in the Indian sector of the Antarctic and in the Weddell Sea during “Research Program of the Russian Federation in Subarea 48.5 (Weddell Sea) in season 2012-13” (Fig. 1; Petrov and Gordeev 2015). Twenty-four specimens of teleost and elasmobranch fish belonging to seven species (Table 1) were examined and found infected by *O. antarcticum*. Coordinates and depths of the catching and the characteristics of the examined fish are given in Table 1.

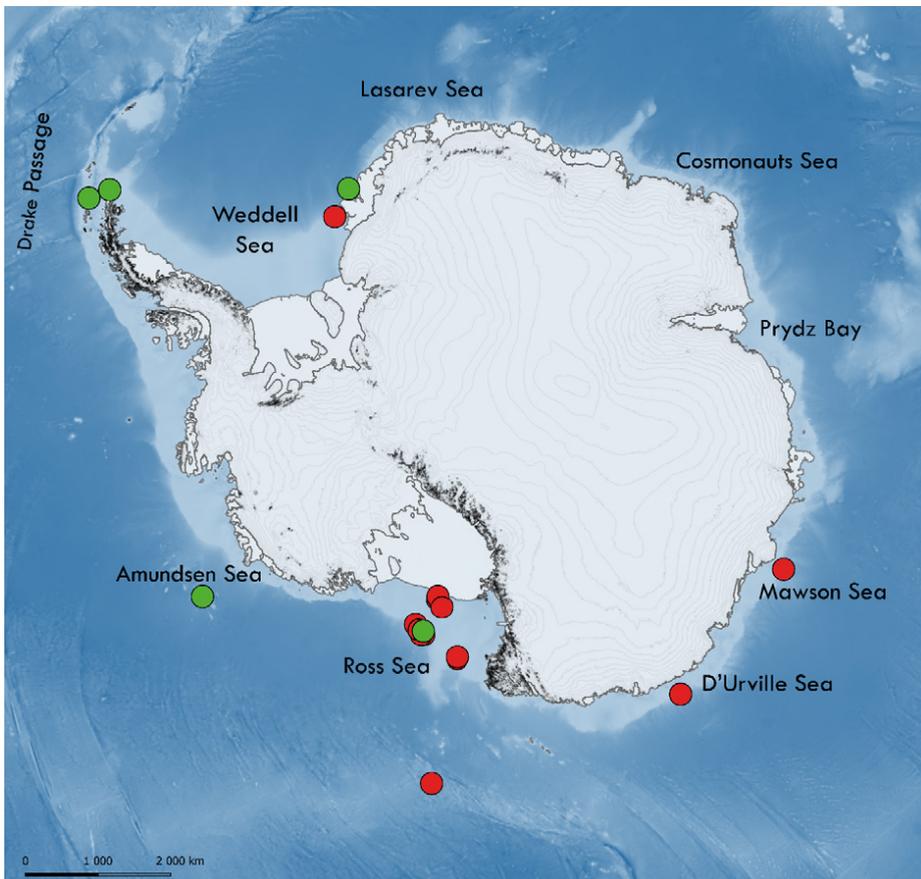


Fig. 1. Spatial distribution of *O. antarcticum* in the Antarctic. Green points – previous records, red – records made in this study.

Table 1

Information about the studied elasmobranch and teleost fish, infected by *O. antarcticum*

Species	Host			Catch information				
	No	Total length (cm)	Weight (kg)	Location	Date	Latitude	Longitude	Depth (m)
<i>Amblyraja georgiana</i>	1	102	8.16	Ross Sea	18.01.2013	75°45'6S	172°28'1W	1110
	2	60	2.04		31.01.2015	75°32'9S	173°22'4W	1268
<i>Bathyraja eatonii</i>	3	95	9.76	Ross Sea	24.12.2011	77°47'9S	178°18'6W	709
	4	105	4.85		24.12.2011	77°47'9S	178°18'6W	709
	5	107	10.46		04.01.2013	73°43'1S	176°38'1E	605
<i>Bathyraja maccaini</i>	6	90	8.14	Ross Sea	04.01.2013	73°43'1S	176°38'1E	605
	7	96	8.18		01.01.2015	77°05'6S	179°36'W	739
<i>Antimora rostrata</i>	8	63	2.16	Ross Sea	03.01.2013	73°36'2S	176°40'2E	602
	9	60	1.54	Weddell Sea	27.02.2013	74°37'1S	28°10'5W	1109
<i>Chionobathyscus dewitti</i>	10	37	0.44	Mawson Sea	10.02.2011	64°58'0S	114°07'0E	1450
	11	38	0.32	Ross Sea (north)	07.12.2014	65°21'2S	178°13'6W	1905
<i>Dissostichus mawsoni</i>	12	144	51.50	D'Urville Sea	10.01.2011	65°29'0S	139°26'0E	810
	13	132	27.20		21.12.2011	77°38'8S	178°06'3W	689
	14	120	17.60		21.12.2011	77°42'4S	178°03'3W	693
	15	144	45.00		09.01.2012	75°11'3S	174°14'3W	1531
	16	105	16.00		12.01.2012	75°25'3S	174°32'7W	1121
<i>Muraenolepis marmorata</i>	17	82	6.30	Ross Sea	04.01.2013	73°43'1S	176°38'1E	605
	18	129	26.00	21.01.2013	75°45'1S	172°42'1W	1071	
	19	102	14.00	21.01.2013	75°45'1S	172°42'1W	1071	
	20	157	51.00	22.01.2013	75°42'2S	172°58'4W	1089	
<i>Muraenolepis marmorata</i>	21	48	0.57	Ross Sea	27.12.2011	75°11'5S	174°54'4W	1463
	22	54	1.28		28.01.2015	75°51'5S	172°21'2W	898
	23	46	0.72		31.01.2015	75°32'9S	173°22'4W	1268
	24	55	0.75		Weddell Sea	03.03.2013	74°36'7S	28°28'9W

Species identification of teleosts and elasmobranchs was based on specialized literature (Gon and Heemstra 1990; CCAMLR 2011). All specimens were dissected immediately after capture using standard methods (Bykhovskaja-Pavlovskaja 1985; Klimpel *et al.* 2019). Specimens for genetic studies were fixed in 96% ethanol and stored at -20 °C. The worms for morphological identification were fixed in 70% ethanol, hydrated, stained with Harris's hematoxylin, differentiated in tap water, destained in ethanol, dehydrated, cleared in methyl salicylate (following Jensen *et al.* 2011), and finally mounted in Canada balsam. The intensity of infection (Bush *et al.* 1997) was roughly estimated, visually, without using a stereomicroscope.

**DNA extraction, amplification, sequencing, alignment and phylogenetic analysis.** — The total DNA was extracted from 96% ethanol-fixed 9 specimens of *O. antarcticum* using Wizard SV Genomic DNA Purification System (Promega), as recommended by the manufacturer. The nuclear 28S rRNA gene was amplified using the polymerase chain reaction (PCR) with the primers ZX-1 (5'-ACCCGCTGAATTTAAGCATAT-3'), 1500R (5'-GCTATCCTGAGG-GAAACTTCG-3'), LSU\_300F (5'-CAAGTACCGTGAGGGAAAGTTG-3'), 1090F (5'-TGAAACACGGACCAAGG-3'), LSU\_1200F (5'-CCCGAAA-GATGGTGAACATATGC-3'), ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3'), which were described earlier (Waeschenbach and Littlewood, 2017). The *cox1* of the same specimens was amplified using the polymerase chain reaction (PCR) with the primers PBI-cox1F\_PCR (5'- CATTGCTGCCGGTCAR-CAYATGTTYTGRTTTTTTGG-3'), PBI-cox1R\_PCR (5'- CCTTGTGCGA-TACTGCCAAARTAATGCATDGGRAA-3'), which were described by Waeschenbach and Littlewood (2017). The initial PCR was performed in a total volume of 20 µl that contained 0.25 mM of each primer pair, 1 µl DNA in water, 1× Taq buffer, 1.25 mM dinucleotide triphosphates (dNTPs), 1.5 mM MgCl<sub>2</sub> and 1 unit of Taq polymerase. The amplification was carried out by Eurogen (Moscow) with a 3-min. denaturation hold at 94°C, 40 cycles of 30 s at 94°C, 30 s at 55°C (*cox1* – 60°C) and 2 min. (*cox1* – 1 min) at 72°C, and a 10-min. extension hold at 72°C. Negative and positive controls were amplified using all primers. The PCR products were directly sequenced using the ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit, as recommended by the manufacturer, with the PCR primers for 28S and with sequencing primers PBI-cox1F\_seq (5'-CATTGCTGCCGGTCA-3'), PBI-cox1R\_seq (5'-TAATGCATDGGRAAAAAC-3') for *cox1* (Waeschenbach and Littlewood 2017). The PCR products were analyzed by Eurogen (Moscow).

**Alignment and phylogenetic analysis.** — Partial sequences used in our study to evaluate the phylogenetic connections of the specimens were assembled using Geneious ver. 10.0.5 software and aligned with sequences retrieved from Genbank (Table 2) using ClustalW DNA weight matrix within MEGA 10.0.5 software alignment explorer (Kumar *et al.* 2018). Phylogenetic analysis of the nucleotide sequences was performed using maximum likelihood (ML) and

Table 2  
 Data on the 28S rDNA and cox1 sequences used in the maximum likelihood and Bayesian inference phylogenetic analysis.

Taxon	GenBank Acc. No.		Host	Location	Reference
	IstDNA	cox1			
<i>Onchobothrium antarcticum</i> adult	MW548862	MW559730	<i>Bathyraja eatonii</i> * <sup>3,4,5,5</sup>	Ross Sea	this study
	MW548863	MW555790			
	MW548955	MW559732			
	MW549040	MW559796	<i>Bathyraja maccaini</i> * <sup>6</sup>		
	MW548961	MW559566			
		KF573588			
<i>Onchobothrium antarcticum</i> larvae		KF573596	<i>Bathyraja eatonii</i>	Bransfield Strait	Laskowski and Rocka (2014)
	KF882019		<i>Notothenia rossii</i>	Bransfield Strait	Laskowski and Rocka (2014)
	KF882020				
	KF882021				
		KF573589			
		KF573590			
		KF573591			
		KF573592			
	KF573594				

Taxon	GenBank Acc. No.		Host	Location	Reference
	1srDNA	cox1			
<i>Onchobothrium antarcticum</i> larvae		KF573595	<i>Notothenia rossii</i>	Bransfield Strait	Laskowski and Rocka (2014)
		KF573597			
		KF573599			
		KF573600			
		MW555776	<i>Dissostichus mawsoni</i> *17,20	Ross Sea	this study
		MW548985			
	MW555788	<i>Chionobathyscus dewitti</i> *11			
	MW549053	<i>Muraenolepis marmorata</i> *22,23			
	MW549201				
<i>Onchobothrium</i> sp. larva		KF573593	<i>Notothenia rossii</i>	Bransfield Strait	Laskowski and Rocka (2014)
<i>Onchobothrium</i> sp. adult	MW566787	MW560094	<i>Bathyraja sexoculata</i>	off Simushir Island	this study
<i>Acanthobothrium rodmani</i>	FJ843596		<i>Himantura</i> sp.	Australia	Fyler <i>et al.</i> (2009)
<i>Acanthobothrium romanowi</i>	FJ843598		<i>Himantura</i> sp.	Australia	Fyler <i>et al.</i> (2009)
<i>Acanthobothrium parviuncinatum</i>	EF095264		<i>Urobatis maculatus</i>	Mexico	Waeschenbach <i>et al.</i> (2007)
<i>Uncibilocularis okei</i>	KF685777		<i>Pastinachus atrus</i>	Australia	Caira <i>et al.</i> (2014)

Taxon	GenBank Acc. No.		Host	Location	Reference
	1stDNA	cox1			
<i>Potamoxygonocestus</i> cf. <i>fitzgeraldae</i>	KF685773		<i>Potamoxygon castexi</i>	Peru	Caira <i>et al.</i> (2014)
<i>Megalonchos shawae</i>	KF685764		<i>Hemipristis elongata</i>	Australia	Caira <i>et al.</i> (2014)
<i>Calliobothrium</i> cf. <i>verticillatum</i>	KF685753		<i>Mustelus canis</i>	USA	Caira <i>et al.</i> 2014
<i>Platybothrium</i> <i>auriculatum</i>	KF685898		<i>Prionace glauca</i>	USA	Caira <i>et al.</i> (2014)
<i>Platybothrium</i> <i>jondoeorum</i>	KF685772		<i>Negaprion acutidens</i>	Australia	Caira <i>et al.</i> (2014)
<i>Proteocephalus</i> <i>macrocephalus</i>	EF095261		<i>Anguilla anguilla</i>	UK	Waeschenbach <i>et al.</i> (2007)
<i>Proteocephalus</i> <i>luciopecae</i>		MN061843	<i>Sander vitreus</i>	Canada	Scholz <i>et al.</i> (2019)
<i>Proteocephalus</i> <i>longicollis</i>		MN061852	<i>Sander vitreus</i>	USA	Scholz <i>et al.</i> (2019)
<i>Proteocephalus pearsei</i>		MN061845	<i>Perca flavescens</i>	USA	Scholz <i>et al.</i> (2019)
<i>Proteocephalus pinguis</i>		MN061849	<i>Esox lucius</i>	USA	Scholz <i>et al.</i> (2019)
<i>Hydatigera</i> sp.		LC008533	<i>Felis catus</i>	France	Lavikainen <i>et al.</i> (2016)
<i>Taenia crocutae</i>		AB905201.1	<i>Crocota crocuta</i>	Africa	Terefe <i>et al.</i> (2014)

(\*) the number(s) after correspond the host number in Table 1 (consistently).

Bayesian (BI) methods. Phylogenetic trees made with the use of ML and BI were reconstructed using MEGA 10.0.5 (Kumar *et al.* 2018) and MrBayes v. 3.6.2 software (Ronquist and Huelsenbeck 2003), respectively. Pairwise distances were calculated using MEGA 10.0.5. Best nucleotide substitution model for the dataset was estimated using jModelTest version 0.1.1 software (Posada 2008). In both methods, the general time-reversible model GTR+G+I was used based on the Aikake Information Criteria (AIC). A Bayesian algorithm was performed using the Markov chain Monte Carlo (MCMC) option. The burnin values were 2,500,000 for the ‘sump’ and the ‘sumt’ options. The robustness of the phylogenetic relationship was estimated using bootstrap analysis with 1000 replications (Felsenstein 1985) for ML and with posterior probabilities for BI (Ronquist and Huelsenbeck 2003). The choice of outgroups generally followed the current phylogeny of cestodes by Caira and Jensen (2017). The obtained sequences of *O. antarcticum* were submitted to GenBank, with accession numbers given in Table 2. Besides the sequences from GenBank and the newly obtained 16 sequences of *O. antarcticum* specimens from the Ross Sea, we involved in the analysis a sequence of *Onchobothrium* sp. collected from *Bathyraja sexoculata* Misawa, Orlov, Orlova, Gordeev *et* Ishihara, 2020 caught off Simushir Island in the northwestern Pacific (see Gordeev and Polyakova 2020; Misawa *et al.* 2020).

## Results

Intestines and pyloric caeca of *Dissostichus mawsoni*, *Chionobathyscus dewitti* Andriashev *et* Neyelov, 1978, *Antimora rostrata* (Günther, 1878), and *Muraenolepis marmorata*, Günther, 1880 were infected with plerocercoids of *O. antarcticum*. Spiral intestines of *Amblyraja georgiana* (Norman, 1938), *Bathyraja maccaini*, and *Bathyraja eatonii* were infected with adults of *O. antarcticum*.

Exact values of infection indices were not determined. We can only give a rough estimation of the intensity of infection. It did not exceed several tens of worms in most fish, but could reach two hundreds of worms and more in large rays. The infection with cestodes was mainly represented by small plerocercoids with bilocular bothridia of an unknown species. The larvae of *O. antarcticum*, which could be easily distinguished by a larger size and trilocular bothridia, were less common. To note, the examined teleost fishes did not seem to be more heavily infected than those examined in our previous study, where about a hundred plerocercoids per one specimen of *D. mawsoni* were found (Gordeev and Sokolov 2016).

Our data show that in the Ross Sea this species occurs not only in the shelf area but also in intrashelf depressions and in submarine elevations in the northern part of the sea (Fig. 1). This is the first record of this tapeworm in the Indian sector of the Antarctic.

Phylogenetic analysis based on 28S gene (Fig. 2) showed that *Onchobothrium* formed a highly supported clade, separated from the other representatives of Onchoproteocephalidea included in the analysis. It also revealed that only three of our sequences were identical with the sequences of *O. antarcticum* obtained by Laskowski and Rocka (2014), which formed subclade B. The rest of our specimens fell into subclade A, which was distinguished by one nucleotide substitution (A/T) in both ML and BI analyses. *Onchobothrium* sp. ex *Bathyraja sexoculata* was different in two loci and was clearly separated from *O. antarcticum* on the tree (Fig. 2).

The *cox1* analyses of all currently available sequences (GenBank) revealed similar results. Despite a high diversity of the *cox1* gene in this species, the topology of the *Onchobothrium* clade was similar to that on the 28S tree. The same isolates that formed subclade A in Fig. 2 fell into clade A on the *cox1* tree. Moreover, specimens of the subclade B clustered with the sequences of *O. antarcticum* obtained by Laskowski and Rocka (2014) in the clade B (Fig. 3). Only MW549201 stands out because we failed to obtain *cox1* of this specimen (see Table 2). Pairwise distances between the members of Clade A and Clade B varied from 5 to 7%.

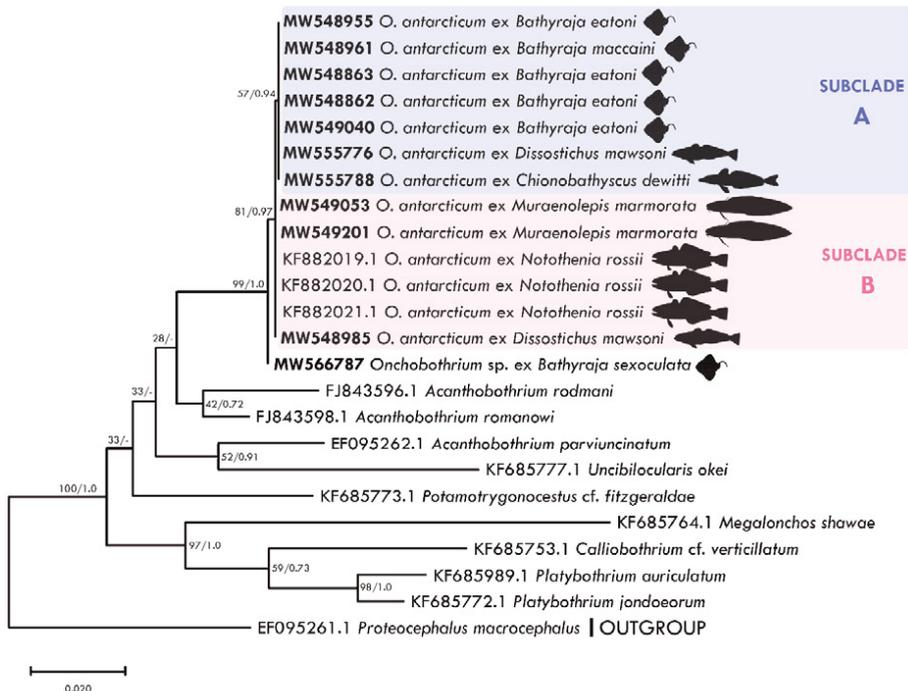


Fig. 2. Phylogenetic position of *Onchobothrium antarcticum* and *Onchobothrium* sp., based on the analysis of 28S rRNA gene partial sequences (1459 bp). Nodal numbers are posterior probability values for bootstrap values for ML/BI. Sequences obtained during this study are in bold.

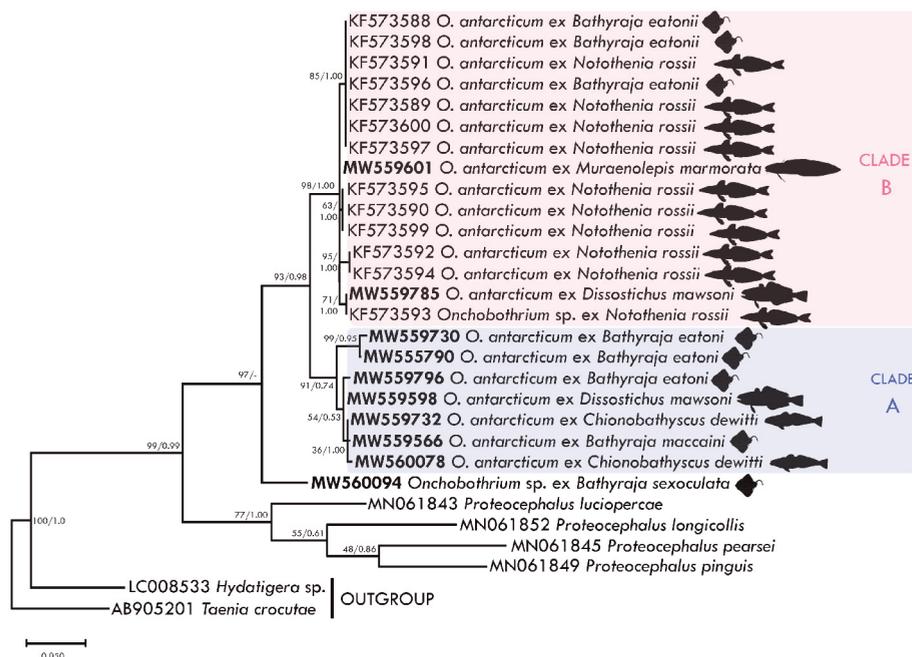


Fig. 3. Phylogenetic position of *Onchobothrium antarcticum* and *Onchobothrium* sp., based on the analysis of *cox1* gene partial sequences (544 bp). Nodal numbers are posterior probability values for bootstrap values for ML/BI. Sequences obtained during this study are in bold.

## Discussion

The topology of 28S and *cox1* trees suggests that the parasite specimens examined in our study could belong to two closely related species of *Onchobothrium*. Unfortunately, this issue cannot be fully elucidated because sequences of larvae and adults mostly grouped into different clades. On the 28S tree (Fig. 2) all sequences of larvae, obtained by Laskowski and Rocka (2014), grouped into subclade B while all adult sequences obtained in our study grouped into another subclade (A). On the *cox1* tree (Fig. 3), three sequences of adults, obtained by Laskowski and Rocka (2014) (KF573588, KF573596, KF573598), showed a well-supported close relation only to the larval sequences in clade B, while four sequences of adults from our study (MW559730, MW555790, MW559796, MW559566) clustered in clade A (Fig. 3).

We do not draw any taxonomic conclusions based on the values of genetic differentiation obtained in this study, given a smooth topology of the *Onchobothrium* clade on the 28S tree, the absence of proven differences in host specificity, site of infection or geographical range (see Table 1, Fig. 1) and the lack of molecular data for other six valid *Onchobothrium* spp. (Caira and Jensen 2017). A careful comparison of the morphology of gravid proglottids of

our specimens with the original description (Wojciechowska 1990a) should be performed in the future in order to ascertain whether our specimens of Clade A belong to a closely related undescribed species.

*Onchobothrium* sp. from *B. sexoculata* collected by us in the northwestern Pacific (MW566787) falls within the *Onchobothrium* clade in Fig. 2. However, it is separated from all the other members of this clade sequenced in this study with a high support and has unique morphological features. Therefore, we conclude that it is probably a new species to describe in the future.

All intermediate and final hosts of *O. antarcticum* are relatively common in the Antarctic and partly the Subantarctic waters (Fig. 1), thus we assume that the spatial distribution of this cestode in the Antarctic is circumpolar. This is typical of many members of the Antarctic marine ecosystem, which is known for its homogeneity (Eastman 1993; Mogue *et al.* 2014; Gordeev 2015). At the same time, studies of migration of the Antarctic toothfish have shown that after leaving the shelf, where their larvae develop, and after the transition from the target feeding on plankton to feeding on fish and molluscs, they almost do not migrate any more (Hanchet *et al.* 2015). Parasites obviously move with their hosts, but taking into account that all fish specimens in this study were caught at a wide range of depths, from 602 m on the shelf to 1905 m, it appears that the current system, including the Antarctic Circumpolar Current, is conducive to the successful dispersal of coracidia and planktonic crustaceans, which act as the first intermediate hosts of cestodes (Marcogliese 1995). The host specificity of *O. antarcticum* seems to be very low both at the level of the definitive and the intermediate host, since it was found in the spiral valves of all studied elasmobranchs and four common teleosts from various taxa.

*Muraenolepis marmorata* from the Ross Sea was examined for the presence of helminths (Gordeev and Sokolov 2017), but only the larvae of Diphyllobothriidae and genus *Calyptrobothrium* were found. It is likely that the larvae of *O. antarcticum* were found in the intestines of this gadid fish in the Ob Bank, the Lena Bank, and in the waters near Kerguelen Island by other authors, listed in Gordeev and Sokolov (2017). However, we cannot be certain about it because authors only identified parasites to a high taxonomic level and provided no morphological descriptions.

Blue hake, *Antimora rostrata* is distributed almost worldwide, inhabiting all ocean waters except the North Pacific, where the congener, the Pacific flatnose *Antimora microlepis* Bean, 1890, occurs. From the previous studies on its infection recorded, mostly in the North Atlantic (Gordeev *et al.* 2017, 2019), *A. rostrata* harbors few or no specialist parasites and is usually involved in the cycles of the local parasite fauna. The only record on its infection by digenean *Elytrophalloides oatesi* (Leiper *et Atkinson*, 1914) in the Subantarctic was made by Gaevskaya and Rodjuk (1988) in the Falkland Islands area. Thus, in this study, we made the first record of helminths of *A. rostrata* from the high latitudes of the Antarctic.

Among all teleost hosts, only *D. mawsoni* could successively serve as the second intermediate host, the paratenic host, and a dead-end host of *O. antarcticum*. After feeding on plankton for some time, it proceeds to feeding on fish and squids. The Antarctic toothfish grows to a length of more than two meters and is one of the highest-order predators. After reaching one meter in length, it can hardly fall prey to rays, even large ones, and can only be consumed by killer whales and other large marine mammals (Yukhov 1982). In our previous study focused on the Antarctic toothfish (Gordeev and Sokolov 2016), the maximum intensity of infection by *O. antarcticum* reached 108 worms and 471 plerocercoids with bilocular bothridia per host, which means that the plerocercoids of *O. antarcticum* probably pass through the food chain and accumulate in the intestines of high-order predators.

*Chionobathyscus dewitti* (Channichthyidae) is a rarely studied deep-water demersal fish. It has been noted as the host of the digenean *Neolepidapedon trematomi* Prudhoe *et* Bray, 1973 (Sokolov and Gordeev 2013). Here we reported for the first time its infection with cestodes. Cestode species identification is difficult because no molecular data on cestode larvae from the Antarctic waters are available. A recent detailed study of five channichthyid species in the north-west Antarctic Peninsula area (Kuhn *et al.* 2018) contains some data on cestode larvae but all of them were identified as “Diphyllobothriidea indet.” or “Tetraphyllidae indet.”, and comparison is thus impossible.

The taxonomy of Antarctic rays needs revision. Most researchers identifying ray species within the framework of the CCAMLR Scheme of International Scientific Observation (SISO) rely on the methodology presented in the Scientific Observer’s Manual (CCAMLR 2011) that base on Gon and Heemstra (1990) and Fischer and Hureau (1985). New genetic data (Smith *et al.* 2008; Stehmann *et al.* 2021) show that *B. eatonii* caught at the continental shelf (Ross Sea) and the Antarctic slope is distinct from *B. eatonii* from the Kerguelen Plateau (type locality). This means that the rays from the Ross Sea, including the host rays in our study, identified by Smith *et al.* (2008) as *Bathyraja* cf. *eatonii*, could belong to another species. The exact distribution of the examined host species is described in different ways in the literature. McCain’s skate *Bathyraja maccaini* appears to have a circum-Antarctic distribution according to Duhamel *et al.* (2014), but according to Last *et al.* (2016) it inhabits only the waters around the tip of the Antarctic Peninsula and off Kerguelen Island. Antarctic starry skate *Amblyraja georgiana* according to Duhamel *et al.* (2014) inhabits the Ross Sea, Amundsen Sea, Bellingshausen Sea, Cooperation Sea and the South Shetland Islands, whereas Last *et al.* (2016) report it occurs mostly off South America and adjacent waters of Pacific and Atlantic sectors of the Antarctic. Unfortunately, although rays are persistent by-catch in longline fisheries for toothfish, the data on their actual distribution obtained by the observers are rarely compiled and made available to the scientific community. To sum up, we are confident about the definition of all the host fish species examined in our study, except that of *B. eatonii*.

## Conclusion

In this study we discovered five new hosts of *O. antarcticum* and added several new geographical records and suggest its circumpolar distribution. Phylogenetic analysis based on 28S and *cox1* genes partial sequences suggests that this species could be a complex of cryptic species. This issue requires a thorough morphological study. Helminth infection of *Antimora rostrata* from the Antarctic was recorded for the first time.

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