

Parasites of five vagrant Polar bears (*Ursus maritimus*) swimming to Iceland during 2008 to 2016

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ABSTRACT

During 2008 to 2016, five polar bears (*Ursus maritimus*) swam from the East Greenland population to Iceland and were shot soon after walking ashore. Each bear was dissected. Ectoparasites were searched for in their ears and fur, helminths in the gastrointestinal tract and *Trichinella* larvae in muscles. Protozoan cysts, oocysts and helminth eggs were also searched for in faecal samples. No ectoparasites were detected. Two bears (40%) hosted fourth stage larvae of the nematode *Contracaecum osculatum* (strain B) in the stomach. Characteristic scars, noted as craters in the rectum wall, indicated previous acanthocephalan infection in one bear. Three polar bears were infected by the nematode *Trichinella nativa*. No protozoan parasites were detected in faecal samples, but cysts of the heterokontophyte *Blastocystis* sp. were found in faeces of two bears (40%). This is the first report of *C. osculatum*, *Blastocystis* sp. and an acanthocephalan infection in free-living polar bears.

Keywords: Iceland, parasites, *Ursus maritimus*, polar bear, vagrant, new host records

YFIRLIT

Sníkjudýr í fimm flækings ísbjörnum (Ursus maritimus) sem syntu til Íslands á árunum 2008 til 2016.

Fimm hvítabirnir úr stofninum við Austur Grænland syntu til Íslands á árunum 2008 til 2016. Birnirnir voru aflífaðir skömmu eftir að hafa stigið hér á land, krufðir og í framhaldinu rannsakaðir í margvíslegu tilliti. Leitað var að útvortis sníkjudýrum í húð, feldi og eyrum. Leitað var að ornum í meltingarvegi og lifrum tríkína (fleskorma) (*Trichinella*) í vöðvum. Í saur var leitað að eggjum sníkjuorma og þolhjúpum einfrumunga. Engin ytri sníkjudýr fundust. Í maga tveggja hvítabjarna (40%) fundust fjórða stigs lifur þráðormsins *Contracaecum osculatum* (strain B). Dæmigerð ör aftast í ristli eins hvítabjarnarins bentu til þess að þar hefðu krókhöfðar áður haldið til - en þeir bora hausnum niður í ristilveginn og orsaka þar dæmigerð hýsilviðbrögð, sem sjást lengi eftir að ormarnir hafa sleppt þar takinu. Þrír hvítabjarnanna voru smitaðir af tríkínum. Við rannsóknir á saursýnunum fundust engin ummerki um smit af völdum einfrumunga í meltingarvegi en þolhjúpur *Blastocystis* sp. (Heterocontophyta) fundust í saur úr tveimur hvítabjarnanna (40%). Þremur þessara tegunda, *C. osculatum*, *Blastocystis* sp. og krókhöfðanum hafði ekki áður verið lýst sem sníkjudýrum villtra hvítabjarna.

INTRODUCTION

The natural habitats of polar bears (*Ursus maritimus*) are found throughout the ice-covered seas of the circumpolar Arctic (Stirling

1998). Polar bears are not native in Iceland, an island located south of the Arctic Circle in the North Atlantic Ocean, between North America

and Europe. However, sightings of polar bears have been documented in Iceland since the 9th century. At least 500 polar bears have reached the island, including 50–60 in the 20th century (Haraldsson & Hersteinsson 2004), a phenomenon that could increase in the future due to global warming, with health consequences for polar bears (Patyk et al. 2015). Usually these polar bears travelled with drifting pack ice to Northwest, North and Northeast Iceland. Some were killed on arrival, but frequently the animals disappeared when they returned back to the drift ice. Some of these accounts referred to bear remains discovered on the shore or even remains that were found in bottom trawls of fishing boats off the coast.

Polar bears are good swimmers and able to travel hundreds of km in a few days without rest (Durner et al. 2011). During 2008 to 2016, five polar bears left their natural distribution area off the coast of East-Greenland and swam to Iceland. At the time of arrival, the East Greenland ice edge was from 110 to 170 km distant from Iceland (Skírnisson, unpublished data). Soon after landing, the bears were shot and subsequently examined for various population and health-related parameters. An extensive dissection report was compiled for each animal and sent to The Environment Agency of Iceland (Skírnisson, unpublished). Various findings based on examinations of these vagrants have been published. Age and life-history traits have been evaluated for each animal by counting and analysing growth layer groups in the cementum region of Incisor 1, as presented for two of the bears in Skírnisson (2009). The identification of *Trichinella nativa* was confirmed at the *International Trichinella Reference Centre* (ITRC) in Roma, Italy by using molecular methods, examining larvae isolated from the first vagrant bear (Male 1, M1), which arrived in Iceland in 2008 (Skírnisson et al. 2010). Later, Skírnisson (2013) reviewed reasons that are believed to explain why Iceland has been free of *Trichinella* infections. Kutschera et al. (2016) included four of the bears in a study of the genetic variability of vagrant polar bears in the range of recognized subpopulations in the northern

hemisphere. McKinney et al. (2011) included samples from a vagrant bear in Iceland in a study of hepatic microsomal biotransformation of selected Polybrominated diphenyl ethers (PBDEs). Levels of organohalogen compounds (PCBs, chlordanes, PBB 153, PBDEs, HCB) have been determined in adipose tissue, liver, kidney and muscle of four polar bears (Vetter et al. 2015). Eibler et al. (2017) analysed lipid classes and fatty acids in malnourished bears. Earlier, Klobes et al. (1998) examined levels and enantiomeric ratios of chlorinated hydrocarbons in the adipose tissue and the liver of a young, adult male killed north of Iceland in 1993, a polar bear that is not included in the results of the present paper.

Wild polar bears, as well as most other carnivores at the top of the food chain in Arctic and subarctic areas of the Holarctic region, are well known to act as reservoir for *T. nativa*, a nematode possessing the important biological characteristic that its larvae survive in frozen carnivore muscles, which favours the transmission from one carnivore host to another under extreme conditions (Rausch 1970, Taylor et al. 1985, Forbes 2000, Rah et al. 2005, Pozio & Murrell 2006). However, little information seems to be available on other parasite species of wild polar bears. Amstrup (2003) did not see any evidence of ectoparasite infestations during 21 years of research in Alaska and concluded that reported diseases and parasites of polar bears were few, with the exception of the two most frequently observed parasites, *Toxoplasma gondii* and *Trichinella* spp. (Fagre et al. 2015, Di Salvo & Chomel 2019, Dubey et al. 2021). Kurnosova et al. (2017) identified helminth eggs and a first stage nematode larva found in faecal samples collected from polar bears along the coast and from the ice surface in the Russian Arctic. Three nematode parasites were found: eggs of *Toxascaris* sp. and of *Uncinaria stenocephala* and first stage larvae identified as *Crenosoma* sp. Eggs of an unidentified trematode (probably Heterophyidae) and eggs of a *Diphyllbothrium* sp. cestode were also identified. There is a possibility that these parasite species appeared in the polar

bear's faeces as the result of consuming an infected arctic fox (*Alopex lagopus*), which is a well known final host of all these parasites (Skirnisson et al. 1993, Meijer et al. 2011).

Some records of polar bear parasites represent accidental infections of species acquired by bears held in captivity. Thus, Rogers & Rogers (1976) enlist three species of nematodes (*Haemonchus contortus*, *Dochmius ursi* and *Baylisascaris transfuga*) and three cestodes (*Diphyllbothrium latum*, *Bothriocephalus* sp. and *Taenia ursi-maritimi*) that have been found in polar bears kept in zoological gardens.

The aim of this article is to present the results of parasitic research carried out on the five vagrant polar bears, one male and four females, especially the discovery of three parasite species that have not been reported previously, and to describe the prevalence and intensity of *T. nativa* infections detected in some of the vagrant polar bears that arrived in Iceland during 2008 to 2016.

MATERIAL AND METHODS

After the polar bears were photographed, weighed, measured, and sexed (Table 1), each bear was examined for ectoparasites. Hair was collected with a comb from different parts of the body and the ears, then examined under a stereomicroscope. After digestion in 10% KOH (Foreyt 2001), samples were also examined under the microscope for the presence of ectoparasites.

During autopsy, inner organs were macroscopically examined for anomalies. Tissue and muscle samples were systematically taken and fixed in 10% buffered formalin. Later, histological sections were prepared, stained with haematoxylin–eosin (H&E) (Suvarna et al. 2013) and examined microscopically for the presence of parasites. The gall bladder was dissected, and its contents examined under a stereoscope. Scrapings from the urinary bladder were examined under the microscope for the presence of worms and helminth eggs.

Stomach, intestine and rectum were separated, with the content of each rinsed under running water into a 10 litres bin filled with approximately 6 litres of water. After thorough mixing and gently stirring, the contents were allowed to sediment for approximately an hour. Helminths were searched for with a stereoscope, both in the floating material and in the sediment.

Unique scars were noticed in the rearmost part of the rectum of Female 3 (F3) post cleansing. They appeared as craters in the mucosa of the rectum wall and were considered to represent previous acanthocephalan infection. The scars were described, counted and photographed.

To evaluate a possible *Trichinella* infection, 50 grams of tongue, diaphragm and masseter, respectively, were weighed from each polar bear and examined by applying the digestion method described by Mayer-Scholl et al. (2017). Intensity of infection is presented as the number of larvae per gram muscle (lpg). Larvae

Table 1. Arrival date and site, length, weight, sex, estimated age and approximate fat proportion of five polar bears (*Ursus maritimus*) shot shortly after having swum to Iceland during 2008 to 2016. Birth month of cubs is set as January.

	Male 1	Female 1	Female 2	Female 3	Female 4
Date of arrival	3 June, 2008	16 June, 2008	27 January, 2010	2 May, 2011	16 July, 2016
Area of Iceland	Middle north	Middle north	Northeast	Westfjords	Middle north
Shooting site	Þverárfell	Hraun á Skaga	Þistilfjörður	Rekavík	Hvalsnes
Total length, cm	209	194	173	173	207
Weight, (kilo)	220	142	138	95	204
Estimated age	22yr, 6 mo	14yr, 6 mo	4 yr, 1 mo	3yr, 5 mo	11yr, 7 mo
Estim. fat (%)	10%	0%	6%	5%	29%

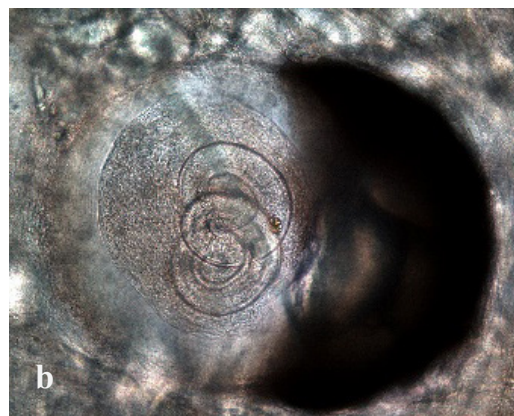


Figure 1 First stage larvae (diameter 40 μm) of *Trichinella nativa* from polar bear (*Ursus maritimus*) Male 1 that swam to Iceland in June 2008. **a.** Free first stage larva from the diaphragm. **b.** Spiral-formed larva encapsulated within a collagenous cyst. The cyst (380 x 500 μm) has a prominent dark-brown lipid droplet at the one end. Photo: Karl Skirnisson.

isolated from M1 (Figure 1, Table 1) were sent to E. Pozio at ITRC in Roma, Italy. There, the identification of *T. nativa* was confirmed by using molecular (PCR) methods (Skirnisson et al. 2010). Unfortunately, later trials to isolate DNA from frozen tissue samples from F2 in Italy were unsuccessful (E. Pozio, personal communication).

Faecal samples were taken from the rectum and examined for the presence of helminth eggs and protozoan cysts or oocysts by using the formalin-ethyl acetate sedimentation technique (FEAST) (Allen & Ridley 1970).

Fourth stage nematode larvae (L4) detected in the stomach of two bears (F3 and F4) were examined morphologically, measured and photographed using a Leica DC 300 digital camera mounted on a Leitz microscope equipped with Nomarski (DIC) contrast. The middle part of one larva found in the stomach of each bear was cut free, fixed in 85% EtOH and preserved for DNA analysis.

After removing ethanol from the nematode samples, DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions. Polymerase

chain reactions (PCR) and sequencing of the D2 domain of the 28S subunit (primers C2'B/D2), the internal transcribed spacers ITS1 and ITS2 (primers BD1/BD2 and C1A/ITS3) of rDNA, and *Cox1* domain (primers JB3/JB4.5) of the mtDNA were performed under the conditions previously described (Morgan & Blair 1995, Jouet et al. 2008, 2010). PCR products were directly sequenced in both directions with the primers used for DNA amplification (Genoscreen, France). Sequences were aligned using ClustalW that is included in MEGA 11 software (Tamura et al. 2021), then checked by eye. Sequences obtained from the two larvae were compared with sequences of nematodes available in GenBank.

RESULTS

The vagrant polar bears

Three of the five polar bears arrived in Iceland in June or July after swimming from further north (Table 1). One (F3) arrived on the island in early May. Geographically, these individuals either arrived in the middle of northern Iceland (three bears) or in the Westfjords (one animal). Names of the arrival

sites are given in Table 1. The fifth bear (F2) reached Northeast Iceland in mid winter and is believed to have come from a pack-ice tongue approximately 150 km northwest of the arrival site. As the ice melted, the animal had to swim, but a strong, long lasting, prevailing northwest wind helped in drifting the female to the arrival site.

Female 2 is probably the only one of the five bears that did not voluntarily decide to leave the natural distribution area of polar bears.

Two of the polar bears were young females (3.5 and 4.1 years/month old; cubs are regarded to be born in January). The others were old individuals, and three of the bears were severely malnourished (see Table 1).

Parasites found within the vagrant bears

Two species of nematode parasites were found. *Trichinella* sp. larvae were present in the musculature of three bears. *C. osculatum* was found in the stomach of two bears (Table 2). In addition, scars believed to indicate a previous acanthocephalan infection were noticed in one bear. Faecal sample examinations revealed neither *Toxoplasma* nor helminths infection, but cysts, identified as *Blastocystis* sp., were detected in faeces from two bears. No ectoparasites were observed, and examinations of histological samples did not reveal any parasites.

Trichinella nativa

Three polar bears (M1, F2 and F4) were infected with larvae of *Trichinella* sp.; the larvae examined from M1 were confirmed to be *T. nativa* (Figure 1). The highest lpg value was detected in the oldest animal, M1 (22.6 years old, average lpg value in its tongue, masseter and diaphragm was 6.6). Comparable values were 2.1 lpg for the 11.7-year old female F4 and 0.9 lpg for the 4.1-year-old subadult female F2.

Intensity of infection on three well-known predilection sites in polar bears was evaluated, with the highest larvae density (lpg value) detected in the tongue (average lpg value 4.5, n=3). Comparable lpg values in masseter and diaphragm were 3.0 and 2.1, respectively (Table 2).

Contracaecum osculatum

Live nematode larvae were found to be mixed with mucus from the stomach in two polar bears, with two larvae in each bear (Figure 2, Table 2). The lengths of the larvae were similar, 12.5 and 13 mm in F3; 10 and 14 mm in F4. Morphologically, the larvae appeared to be identical, and all had reached the fourth larval stage, L4. Based on morphological criteria, the larvae belonged to the subfamily Contracaecinae (Anisakidae). Due to the difficulties in distinguishing the morphologically similar L4 larvae of *C. osculatum* and *Phocascaris* spp. (Berland 1964, Fagerholm 1982), a molecular

Table 2. Parasites of five vagrant Polar bears (*Ursus maritimus*) that swam to Iceland in the period from 2008 to 2016. Numbers of larvae per gram musculature (lpg) are given for polar bears infected by *Trichinella nativa*, and numbers of fourth stage *Contracaecum osculatum* larvae (larval stage 4, L4) found in the stomach of two bears are reported.

	Male 1	Female 1	Female 2	Female 3	Female 4
<i>Trichinella nativa</i>					
in tongue, lpg	8.5	0	1.2	0	3.7
in masseter, lpg	6.8	0	1.0	0	1.3
in diaphragm, lpg	4.4	0	0.6	0	1.4
<i>Contracaecum osculatum</i> (L4)	0	0	0	2	2
Acanthocephala - signs in rectum	-	-	-	+	-
<i>Blastocystis</i> sp. - cysts in faeces	-	-	-	+	+



Figure 2 Fourth stage larvae (L4) of *Contacaecum osculatum* from the stomach of polar bear (*Ursus maritimus*) Female 3. **a.** Two free-moving larvae; **b.** Anterior end with anterior caecum and posterior appendix; **c.** Anterior end showing lips and shape of head; **d.** Shape of the tail. Scale bars **a** 2 mm, **b** 100 µm, **c,d** 250 µm. Photo: Karl Skírnisson.

analysis was carried out to confirm the genus/species of the isolated larvae. The two available nematode samples (CON1 from the stomach of F3 and CON2 from F4) were analysed (D2, ITS1, ITS2, *Cox1*), and then compared with all sequences available in GenBank (3 sequences for *Phocascaris* spp. and approximately 2000 sequences for *Contracaecum* spp., including 324 sequences of *C. osculatum*).

The CON1 and CON2 sequences were 100% homologous for D2 (401 bp) with haplotype Acc. Ns AF226580, an adult of *C. osculatum* strain B (sensu Nascetti et al. 1993), which had been isolated from a harp seal (*Phoca groenlandica*) from Canada by Nadler et al. (2000). For ITS2, CON1 and CON2, sequences were 100% homologous with haplotypes Acc. Ns. MG787557, corresponding

to *C. osculatum* isolated from Atlantic cod (*Gadus morhua*) from Poland (Liu & Liu 2018, direct submission) and Acc. Ns. AJ250420, corresponding to an adult of *C. osculatum* strain B isolated from a harp seal from Atlantic Canada (Zhu et al. 2000). For ITS1, only a few intraspecific variations (9 and 17 out of 434 bp) were observed between CON1, CON2 and sequences Acc. Ns. AJ225062 and KM273050, corresponding to larvae of *C. osculatum* isolated from Atlantic Cod from Gdansk Basin in Poland (Zhu et al. 1998) and from Denmark (Mehrdana et al. 2014). Finally, only intraspecific variations (5 to 7 out of 366 bp) were observed for *Cox1* between our sequences and larvae of *C. osculatum* strain B sensu Nascetti et al. (1993) (Acc. Ns. JN786333 and JF711063-JF711066) isolated from Greenland halibut (*Reinhardtius*

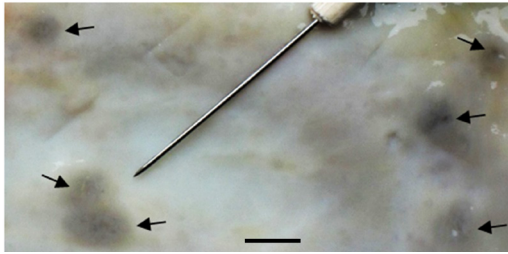


Figure 3 Six crater-like, grey-coloured scars in the mucosa of the hindmost part of the rectum of polar bear (*Ursus maritimus*) Female 3. Each scar (black arrows) is believed to represent previous attachment location of an acanthocephalan, the site where the worm had embedded its proboscis into the rectum wall. Dark spots in the centre of some scars are believed to represent haemorrhagic lesions caused by thorns of the proboscis. Scale bar 500 μm . Photo: Karl Skirnisson.

hippoglossoides) in the Barents Sea (Dzido et al. 2011, direct submission; Dzido et al. 2012).

The results confirmed the membership of our samples to the “*C. osculatum* complex,” which includes six sibling species: four representing the Arctic members (*C. osculatum* strain A, *C. osculatum* strain B, *C. osculatum* sensu stricto, *C. osculatum baicalensis*), and two representing Antarctic specimens (*C. osculatum* strain D, *C. osculatum* strain E), and that the larvae in our samples belonged to the species *C. osculatum* strain B sensu Nascetti et al. (1993).

Acanthocephala

Nine greyish, crater-formed protrusions, some having a dark spot in the centre, were observed on the mucosa of the rearmost 10 cm of the rectum of the subadult female F3 (Figure 3). Comparable lesions were not noted in the other animals. Diameter of each protrusion varied between 1.5 and 2.0 mm. Each such scar is believed to represent an earlier infection by an acanthocephalan that had inserted its proboscis into the rectum wall, causing inflammation and the observed host reaction.

Blastocystis sp.

Examination of faecal samples with the sedimentation technique did not reveal any protozoan infections. However, cysts of *Blastocystis* sp. were found in faeces of two bears (Figure 4, Table 2). By microscopic examination of the sediment, approximately 200 cysts (F3) and around 40 cysts (F4) were counted on a slide with a 20 x 20 mm cover glass. Diameter of the spherical cysts varied between 7 and 9 μm . Light microscopy examination of the cysts indicated a central vacuole that appeared to be surrounded by a thin band of peripheral cytoplasm, which contained other organelles.

The cyst wall thickness varied between approximately 0.7 and 1.0 μm . Most cysts were thin-walled (Figure 4a,c); thick-walled cysts (Figure 4b) were rarely noticed in F4.

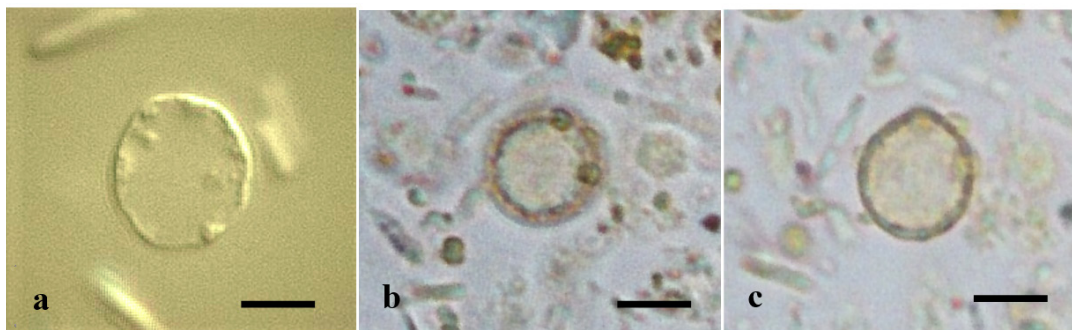


Figure 4. *Blastocystis* sp. cysts detected in faeces of two polar bears (*Ursus maritimus*) (Female 3 and Female 4) that swam to Iceland in 2011 and 2016, respectively. Note the characteristic refractile cyst wall surrounded by loose irregular outer coat: **a**. Cyst from Female 3, **b,c** two cysts from Female 4, note the different thickness of the cyst wall. Scale bars **a,b,c** 5 μm . Photo: Karl Skirnisson.

DISCUSSION

The study and identification of pathogens is essential for understanding the impact of climate change on the health of wildlife. Some threats and metrics have thus been defined, including the search for parasites in polar bears (Fagre et al. 2015). Indeed, the displacement of populations induces exposure with new parasites, having an impact on the health and survival of the animal, but also inducing a risk of zoonoses for humans (Fagre et al. 2015, Patyk et al. 2015, Atwood et al. 2017).

Wild polar bears are well known hosts of *Trichinella nativa* (Rausch 1970, Forbes 2000, Pozio & Murrell, 2006, Seymour et al. 2014, Crisóstomo-Jorquera & Landaeta-Aqueveque 2021) and *Toxoplasma gondii* (Fagre et al. 2015, Dubey et al. 2021). However, according to the available literature, *Contracaecum osculatum* nematodes in the stomach, cysts believed to belong to *Blastocystis* sp. in faeces, and scars believed to represent earlier acanthocephalid infection in the rectum, have not previously been reported from wild polar bears.

Various reasons have been suggested to explain the lack of diversity within the parasite fauna of wild polar bears living on ice. Certainly, limited research could be a part of the explanation, but solitary lifestyle with restricted social encounters (Stirling 1988) and lack of suitable intermediate or definitive hosts for the maintenance of complicated life cycles out on the ice are likely to contribute to the low parasitic burden observed. By contrast, in the review of Rogers & Rogers (1976), 77 species of parasites have been reported for wild and captive ursine bears in terrestrial ecosystems - 15 parasites (including trematodes, cestodes, nematodes and arthropods) are known from brown-grizzly bears (*Ursus arctus*) in North America, 11 of these have also been reported from wild black bears (*Ursus americanus*). In Eurasia, 28 parasite species have been reported from brown bears (*U. arctus*).

Recent examination of faeces of polar bears living along or near the coast in the Russian Arctic revealed five helminth species that have all been reported to be common parasites of

the arctic fox (Skirnisson et al. 1993, Meijer et al. 2011), which might have been acquired by scavenging fox cadavers (Kurnosova et al. 2017) or consuming their faeces. However, it should be noted that, e.g., the nematodes *Uncinaria stenocephala* and *Toxascaris leonina* have a wide range of potential mammalian definitive hosts and may be naturally occurring parasites of polar bears, especially in areas where direct life cycles might be maintained.

Trichinella and their absence in Iceland

T. nativa is widespread in Arctic and subarctic areas of the Holarctic region. Carnivores at the top of the food chain, mainly the polar bear and arctic fox, act as a reservoir, but a number of omnivorous and even herbivorous mammals can also become infected. In Europe some countries (e.g. Denmark and Luxembourg) and islands (e.g. the British Isles, Sardinia, Sicily, Malta and Cyprus) are free of *Trichinella* spp. (Rausch 1970, Taylor et al. 1985, Pozio & Murrell 2006). Iceland is also one of the *Trichinella*-free islands. Systematic search for *Trichinella* larvae in domestic pigs in the 1950s and examination of feral minks (*Neovison vison*) in 1993 did not reveal *Trichinella* infections (Skirnisson et al. 2010). Furthermore, no larvae were found in more than 8.000 horses examined during 1998 to 2012 from all parts of Iceland. Additionally, in 2012 more than 60.000 domestic pigs were found to be free of a *Trichinella*-infection (Skirnisson et al. 2010).

Written sources report more than 500 polar bears to have been recorded in Iceland since the 9th century. Since 2008, three polar bears have been reported to be infected with *Trichinella* sp. in Iceland. In one of the cases *T. nativa* was proven to be the species involved. We consider this species also to have been present in the other two vagrants originating from the East Greenland population. The fourth polar bear known to be infected by *Trichinella* sp. was shot in 1963 (Skirnisson 2013). Based on these findings, and the prevalence of *T. nativa* infections in polar bears in Greenland, we can assume that *T. nativa*-infected polar bears have repeatedly arrived and died in Iceland.

However, no sources indicate that the parasite has ever become indigenous. The few terrestrial mammalian species that could participate in *Trichinella* transmission in Iceland include the arctic fox, mice (mainly *Apodemus sylvaticus*), rats (mainly the brown rat *Rattus norvegicus*) and the feral mink (Skírnisson 2004). Therefore, in order to prevent a possible transmission in Iceland, remains of polar bears should be safely removed and made inaccessible for mammalian scavengers.

The absence of *T. spiralis* and *T. nativa*, however, is partly explained by the fact that domestic pigs had disappeared in Iceland some centuries before the brown rat (these two being the mammals usually needed for a successful transmission of *T. spiralis*) colonized Iceland in the 18th century (Skírnisson 2004, 2013). The isolation of the country and the restricted and controlled importation of potential hosts, along with a scarcity of appropriate hosts favouring a successful *T. spiralis* transmission, certainly contribute to the absence of *T. spiralis* in Iceland.

Born & Henriksen (1990) reported a higher incidence of infection with *Trichinella* sp. with increased age of polar bears. In their studies, no bears younger than three years old were found to be infected, but the prevalence of *Trichinella* sp. among bears of the age group 3–4 years was 25%, and 53% among older animals. Our results are comparable, reporting three out of four bears (75%) that were older than 4 years hosted *Trichinella* sp. However, our finding that lpg values increased with increased host age is probably a coincidence.

Contracaecum osculatum and *Phocascaris* spp. Taxonomic distinction between the two genera (*Contracaecum* and *Phocascaris*) is based on the presence (*Contracaecum*) or absence (*Phocascaris*) of interlabia and a labial denticulation in *Phocascaris* (Berland 1964, Khalil et al. 1994). Molecular analyses showed that the L4 larvae obtained from the stomach of polar bears F3 and F4 were identical and belonged to the species *C. osculatum*. Within this species, the results obtained for D2, ITS1, ITS2 and *Cox1* confirmed that our L4 samples

belonged to the *C. osculatum* clade B *sensu* Nascetti et al. (1990, 1993), a strain also isolated from Atlantic cod, Greenland halibut and harp seal (Zhu et al. 1998, 2000, Nadler et al. 2000, Mattiucci et al. 2008, Dzido et al. 2012, Mehrdana et al. 2014). This clade/strain B, described for the first time by Nascetti et al. (1993), also includes specimens isolated from harp seals, grey seals (*Halichoerus grypus*), common seals (*Phoca vitulina*), bearded seals (*Erignathus barbatus*) and hooded seals (*Cystophora cristata*).

Infective third stage larvae of *Contracaecum* occur in the liver of fish (Fagerholm 1982), with dozens of marine fish species having been reported to act as the second intermediate host in the life cycle (Lick 1991, Hauksson 1992). When consumed with the fish host by mammals (or birds), the L3 larva moults to L4 larvae, which starts its development in the definitive host. Common definitive hosts in the life cycle of *C. osculatum* are the most important prey species of polar bears in the East-Greenland population, namely ringed seals (*Phoca hispida*), and to a lesser degree the bearded seal and other seal species (Stirling 1988), which acquire the infection by eating L3 infected fish.

Two possibilities are regarded to explain the origin of the *C. osculatum* infection in the polar bears. Most likely the larvae (L3 or L4) occurred in the stomach of a seal that the bears had consumed. If this was the case, the seal served as a paratenic host for the larvae that then continued to live in the new host, the polar bear. However, the small size of the larvae (12.5–14.5 mm) suggests either a recent infection or an inadequate developmental environment in the stomach of the bear. However, it cannot be ruled out, although unlikely, that the polar bears acquired the larvae by directly feeding on a L3 infected fish. In this case the larvae would already have developed to the L4 stage in the stomach of the bears.

Acanthocephala

It is unknown which acanthocephalan might have been involved in forming the lesion observed on the mucosa in the rectum. The scars

are considered to have been formed where the proboscis of an acanthocephalan worm had been embedded in the wall, with its body protruding into the rectum lumen. Host reactions and inflammation around the embedding proboscis are regarded to have formed the crater-like protrusions. The dark spots sometimes noticed in the centre most likely represent haemorrhagic lesions caused by thorns of the proboscis that injured the rectum wall.

Corynosoma spp., which frequently parasitize phocid seals (Leidenberger et al. 2020), are likely candidates in this case. Representatives of the genus have a heteroxenous life cycle, comprising a peracaridan intermediate host, a fish paratenic host and a wide range of potential mammalian definitive hosts. Occasionally, *Corynosoma* spp. enter an accidental definitive host, from which it is unable to complete its life cycle (Leidenberger et al. 2020). It seems likely that this happened in the present case, that acanthocephalan worms living in the intestine of a phocid seal had shifted definitive hosts after having been consumed by the polar bears. The scar formation and the prominent host reaction indicate that the acanthocephalans might have been attached to the rectum wall for weeks.

Blastocystis sp.

Blastocystis is an unicellular protist belonging to the eukaryotic phylum Heterokontophyta, which initially was considered to be a commensal, but later observations and studies strongly suggest it to be a pathogen in humans. Although this is supported by strong clinical and scientific evidence, the virulence factors, pathogenicity and other risk factors involved in disease manifestation are still obscure (Parja & Jeremiah 2013). An alternative theory, that *Blastocystis* is not a pathogen at all, has recently been strengthened based on its biochemistry (Stensvold & Giezen 2018).

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REFERENCES

- Allen AV & Ridley DS 1970.** Further observations on the formol-ether concentration technique for faecal parasites. *Journal of Clinical Pathology* 23:545–546.
Doi: [10.1136/jcp.23.6.545](https://doi.org/10.1136/jcp.23.6.545)
- Amstrup SC 2003.** The Polar Bear - *Ursus maritimus*. Biology, Management, and Conservation. In: (eds: Feldhamer GA, Thompson BC & Chapman JA. Wild Mammals of North America. 2nd Edition. John Hopkins University Press, Baltimore. pp. 587-610.
- Atwood TC, Duncan C, Patyk KA, Nol P, Rhyan J, McCollum M, McKinney MA, Ramey AM, Cerqueira-Cézar CK, Kwok OCH, Dubey JP & Hennager S 2017.** Environmental and behavioral changes may influence the exposure of an Arctic apex predator to pathogens and contaminants. *Scientific Reports* 7(1):13193.
Doi: [10.1038/s41598-017-13496-9](https://doi.org/10.1038/s41598-017-13496-9)
- Berland B 1964.** *Phocascaris cystophorae* sp. nov. (Nematoda) from the hooded seal, with an emendation of the genus. *Årbok for Universitetet i Bergen, Series mathematica rerumque naturalium* 17:1–21.
Doi: [10.1023/A:1014083331759](https://doi.org/10.1023/A:1014083331759)
- Born EW & Henriksen AA 1990.** Prevalence of *Trichinella* sp. in polar bears (*Ursus maritimus*) from northeastern Greenland. *Polar Research* 8:313–315.
Doi: [10.3402/polar.v8i2.6824](https://doi.org/10.3402/polar.v8i2.6824)
- Crisóstomo-Jorquera V. & Landaeta-Aqueveque C. 2022.** The genus *Trichinella* and its presence in wildlife worldwide: A review. *Transboundary and Emerging Diseases* 69: e1269– e1279.
Doi: [10.1111/tbed.14554](https://doi.org/10.1111/tbed.14554)
- Di Salvo AR & Chomel BB 2020.** Zoonoses and potential zoonoses of bears. *Zoonoses Public Health* 67:3-13.
Doi: [10.1111/zph.12674](https://doi.org/10.1111/zph.12674)

- Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH & Su C 2021. Epidemiologic and public health significance of *Toxoplasma gondii* infections in bears (*Ursus* spp.): A 50 year review including recent genetic evidence. *Journal of Parasitology* 107:519-528.
Doi: [10.1645/21-16](https://doi.org/10.1645/21-16)
- Durner GM, Whiteman JP, Harlow HJ, Amstrup SC, Regehr EV & Ben-David M 2011. Consequences of long-distance swimming and travel over deep-water pack ice for a female polar bear during a year of extreme sea ice retreat. *Polar Biology* 34:975-984.
Doi: [10.1007/s00300-010-0953-2](https://doi.org/10.1007/s00300-010-0953-2)
- Dzido J, Kijewska A & Rokicki J 2012. Selected mitochondrial genes as species markers of the Arctic *Contracaecum osculatum* complex. *Journal of Helminthology* 86(2):252-8.
Doi: [10.1017/S0022149X11000332](https://doi.org/10.1017/S0022149X11000332)
- Eibler D, Krüger S, Skirnisson K, Vetter W 2017. Combined thin layer chromatography and gas chromatography with mass spectrometric analysis of lipid classes and fatty acids in malnourished polar bears (*Ursus maritimus*) which swam to Iceland. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1046:138-146
Doi: [10.1016/j.jchromb.2017.01.043](https://doi.org/10.1016/j.jchromb.2017.01.043)
- Fagerholm HP 1982. Parasites of fish in Finland VI. Nematodes. *Acta Academiae Aboensis Ser. B. Vol. 40. Nr 6*, 127 p.
- Fagre AC, Patyk KA, Nol P, Atwood T, Hueffer K & Duncan CA 2015. Review of infectious agents in polar bears (*Ursus maritimus*) and their long-term ecological relevance. *Ecohealth* 12:528-539.
Doi: [10.1007/s10393-015-1023-6](https://doi.org/10.1007/s10393-015-1023-6)
- Forbes LB 2000. The occurrence and ecology of *Trichinella* in marine mammals. *Veterinary Parasitology* 93:321-334.
Doi: [10.1016/S0304-4017\(00\)00349-6](https://doi.org/10.1016/S0304-4017(00)00349-6)
- Foreyt WJ 2001. Veterinary parasitology reference manual. 5th ed. Iowa State University Press.
- Haraldsson Þ, Hersteinsson P 2004 [Polar Baer]. In: Hersteinsson P (ed). *Íslensk spendýr. Vaka Helgafell*, Reykjavík. pp. 102-107 (In Icelandic).
- Hauksón E 1992. Hringormasýking nokkurra fiskitegunda við Íslandsstrendur. *Hafrannsóknir* 43:107-121. (In Icelandic).
- Jouet D, Ferté H, Depaquit J, Rudolfová J, Latour P, Zanella D, Kaltenbach ML & Léger N 2008. *Trichobilharzia* spp. in natural conditions in Annecy Lake, France. *Parasitology Research* 103(1):51-8.
Doi: [10.1007/s00436-008-0926-3](https://doi.org/10.1007/s00436-008-0926-3)
- Jouet D, Skirnisson K, Kolářová L & Ferté H 2010. Molecular diversity of *Trichobilharzia franki* in two intermediate hosts (*Radix auricularia* and *Radix peregra*): a complex of species. *Infection, Genetics and Evolution* 10(8):1218-27.
Doi: [10.1016/j.meegid.2010.08.001](https://doi.org/10.1016/j.meegid.2010.08.001)
- Khalil LF, Jones A & Bray RA 1994. Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, UK.
Doi: [10.1016/0169-4758\(95\)80142-1](https://doi.org/10.1016/0169-4758(95)80142-1)
- Klobes U, Vetter W, Glotz D, Luckas B, Skirnisson K & Hersteinsson P 1998. Levels and enantiomeric ratios of chlorinated hydrocarbons in livers of Arctic fox (*Alopex lagopus*) and adipose tissue and liver of a Polar bear (*Ursus maritimus*) sampled in Iceland. *International Journal of Environmental Analytical Chemistry* 69:67-81.
- Kurnosova OP, Khrustalev AV, Illarionova NA & Odoevskaya IM 2017. A survey of helminths of polar bears in the Russian Arctic. *Czech Polar Reports* 7:164-168.
Doi: [10.5817/CPR2017-2-16](https://doi.org/10.5817/CPR2017-2-16)
- Kutschera VE, Frosch C, Janke A, Skirnisson K, Bidon T, Lecomte N, Fain SR, Eiken H-E, Hagen SB, Arnason U, Laidre KL, Nowak C & Hailer F 2016. High genetic variability of vagrant polar bear illustrates importance of population connectivity in fragmented ice sea habitats. *Animal Conservation* 19:337-349. Print ISSN 1367-9430
Doi: [10.1111/acv.12250](https://doi.org/10.1111/acv.12250)
- Leidenberger S, Boström S, Wayland MT 2020. Host records and geographical distribution of *Corynosoma magdaleni*, *C. semerme* and *C. strumosum* (Acanthocephala: Polymorphidae). *Biodiversity Data Journal* Apr 6;8:e50500.
Doi: [10.3897/BDJ.8.e50500](https://doi.org/10.3897/BDJ.8.e50500)
- Lick RR 1991. Untersuchungen zu Lebenszyklus (Krebse – Fische – marine Säuger) und Gefrierresistenz anisakider Nematoden in Nord- und Ostsee. *Berichte aus dem Institut für Meereskunde an der Universität Kiel* Nr. 218. 202p.

- Mattiucci S, Paoletti M, Webb SC, Sardella N, Timi JT, Berland B & Nascetti G 2008.** Genetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Anisakidae) from pinnipeds inferred from mitochondrial *cox2* sequences, and congruence with allozyme data. *Parasite* 15(3):408-419.
Doi: [10.1051/parasite/2008153408](https://doi.org/10.1051/parasite/2008153408)
- Mayer-Scholl A, Pozio E, Gayda J, Thaben N, Bahn P & Nöckler K 2017.** Magnetic Stirrer Method for the Detection of *Trichinella* Larvae in Muscle Samples. *Journal of Visualized Experiments* (121), e55354,
Doi: [10.3791/55354](https://doi.org/10.3791/55354)
- McKinney MA, Dietz R, Sonne C, Guise SD, Skirnisson K, Karlsson K, Steingrímsson E, Letcher R 2011.** Comparative hepatic microsomal biotransformation of selected PBDEs including decabromodiphenyl ether, and decabromodiphenyl ethane flame retardants in Arctic marine-feeding mammals. *Environmental Toxicology and Chemistry* 30:1506-1514.
<https://doi.org/10.1002/etc.535>
- Mehrdana F, Bahloul QZ, Skov J, Marana MH, Sindberg D, Mundeling M, Overgaard BC, Korbut R, Strøm SB, Kania PW & Buchmann K 2014.** Occurrence of zoonotic nematodes *Pseudoterranova decipiens*, *Contracaecum osculatum* and *Anisakis simplex* in cod (*Gadus morhua*) from the Baltic Sea. *Veterinary Parasitology* 15;205(3-4):581-7.
Doi: [10.1016/j.vetpar.2014.08.027](https://doi.org/10.1016/j.vetpar.2014.08.027)
- Meijer T, Mattsson R, Angerbjörn A, Osterman-Lind E, FernándezAguilar X & Gavier-Widén D. 2011.** Endoparasites in the endangered Fennoscandian population of arctic foxes (*Vulpes lagopus*). *European Journal of Wildlife Research*. August 2011.
Doi: [10.1007/s10344-011-0505-2](https://doi.org/10.1007/s10344-011-0505-2).
- Morgan JAT & Blair D 1995.** Nuclear rDNA ITS sequence variation in the trematode genus *Echinostoma*: an aid to establishing relationships within the 37-collar-spine group. *Parasitology* 111, 609-615.
Doi: [10.1017/S003118200007709X](https://doi.org/10.1017/S003118200007709X)
- Nadler SA, D'Amelio S, Fagerholm H-P, Berland B & Paggi L 2000.** Phylogenetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. *Parasitology* 121:455-463.
Doi: [10.1051/parasite/2008153408](https://doi.org/10.1051/parasite/2008153408)
- Nascetti G, Bullini L, Cianchi R, Paggi L, Orecchia P, Mattiucci S, D'Amelio S & Berland B 1990.** Genetic relationships among anisakid species belonging to the genera *Contracaecum* and *Phocascaris*. *Bulletin de la Société Française de Parasitologie* 8 (Suppl. 1), 261.
Doi: [10.1051/parasite/2008153408](https://doi.org/10.1051/parasite/2008153408)
- Nascetti G, Cianchi R, Mattiucci S, D'Amelio S, Orecchia P, Paggi L, Bratney J, Berland B, Smith JW & Bullini L 1993.** Three sibling species within *Contracaecum osculatum* (Nematoda, Ascaridida, Ascaridoidea) from the Atlantic Arctic-Boreal region: reproductive isolation and host preferences. *International Journal of Parasitology* 23(1):105-20.
Doi: [10.1016/0020-7519\(93\)90103-6](https://doi.org/10.1016/0020-7519(93)90103-6)
- Parja SC & Jeremiah SS 2013.** *Blastocystis*: Taxonomy, biology and virulence. *Tropical Parasitology* 3:17-25.
Doi: [10.4103/2229-5070.113894](https://doi.org/10.4103/2229-5070.113894)
- Patyk KA, Duncan C, Nol P, Sonne C, Laidre K, Obbard M, Wiig Ø, Aars J, Regehr E, Gustafson LL & Atwood T 2015.** Establishing a definition of polar bear (*Ursus maritimus*) health: a guide to research and management activities. *The Science of the Total Environment* 514:371-378.
Doi: [10.1016/j.scitotenv.2015.02.007](https://doi.org/10.1016/j.scitotenv.2015.02.007)
- Pozio E & Murrell KD 2006.** Systematics and epidemiology of *Trichinella*. *Advances in Parasitology* 63:367-439.
Doi: [10.1016/S0065-308X\(06\)63005-4](https://doi.org/10.1016/S0065-308X(06)63005-4)
- Rah H, Chomel BB, Follmann EH, Kasten RW, Hew CH, Farver TB, Garner GW & Amstrup SC 2005.** Serosurvey of selected zoonotic agents in polar bears (*Ursus maritimus*). *Veterinary Records* 156:7-13.
Doi: [10.1136/vr.156.1.7](https://doi.org/10.1136/vr.156.1.7)
- Rausch RL 1970.** Trichinosis in the Arctic. In: Gould SE (ed.) *Trichinosis in man and animals*. Springfield, Illinois, Charles C. Thomas Publisher. pp. 348-373.

- Rogers LL & Rogers SM 1976.** Parasites of bears: A review. *International Conference on Bear Research and Management* 3:411–30.
- Seymour J, Horstmann-Dehn L, Rosa C & Lopez JA 2014.** Occurrence and genotypic analysis of *Trichinella* species in Alaska marine-associated mammals of the Bering and Chukchi seas. *Veterinary Parasitology* 200:153–164.
Doi: [10.1016/j.vetpar.2013.11.015](https://doi.org/10.1016/j.vetpar.2013.11.015)
- Skírnisson K 2004** [Mice and rats]. In: Hersteinsson P (ed). *Íslensk spendýr*. Vaka Helgafell, Reykjavík. pp. 262–287 (In Icelandic).
- Skírnisson K 2009.** Age determination and predicted life history of two polar bears *Ursus maritimus* which swam to Iceland in June 2008. *Náttúrufræðingurinn* 78:39–45 [In Icelandic with English summary].
- Skírnisson K. 2013.** On *Trichinella* spp. and their absence in Iceland. *Náttúrufræðingurinn* 83:21–27. [In Icelandic with English summary].
- Skírnisson K, Eydal M, Gunnarsson E & Hersteinsson P 1993.** Parasites of the arctic fox (*Alopex lagopus*) in Iceland. *Journal of Wildlife Diseases* 29:440–446.
Doi: [10.7589/0090-3558-29.3.440](https://doi.org/10.7589/0090-3558-29.3.440)
- Skírnisson K, Marucci G, Pozio E 2010.** *Trichinella nativa* in Iceland: An example of *Trichinella* dispersion in a frigid zone. *Journal of Helminthology* 84:182–185.
Doi: [10.1017/S0022149X09990514](https://doi.org/10.1017/S0022149X09990514)
- Stensvold CR, van der Giezen M 2018.** Associations between gut microbiota and common luminal intestinal parasites. *Trends in Parasitology* 34:369–377.
Doi: [10.1016/j.pt.2018.02.004](https://doi.org/10.1016/j.pt.2018.02.004). hdl:10871/32596. PMID 29567298.
- Stirling I 1988.** Polar Bears. The University of Michigan Press. 220 p.
- Suvarna K, Leyton C & Bancroft JD (eds) 2012.** Bancroft's theory and practice of histological techniques. 7th ed. Churchill Livingstone, Elsevier. ISBN: 9780702042263
- Tamura K, Stecher G & Kumar S 2021.** MEGA11: Molecular Evolutionary Genetics Analysis Version 11, *Molecular Biology and Evolution* 38:3022–3027.
Doi: [10.1093/molbev/msab120](https://doi.org/10.1093/molbev/msab120)
- Taylor MK, Larsen T & Schweinsburg ER 1985.** Observations of intraspecific aggression and cannibalism in polar bears (*Ursus maritimus*). *Arctic* 38:303–309.
- Vetter W, Gall V & Skirnisson K 2015.** Polyhalogenated compounds (PCBs, chlordanes, HCB and BFRs) in four polar bears (*Ursus maritimus*) that swam malnourished from East Greenland to Iceland. *Science of the Total Environment* 533:290–296.
Doi: [10.1016/j.scitotenv.2015.07.011](https://doi.org/10.1016/j.scitotenv.2015.07.011)
- Zhu X, D'Amelio S, Paggi L & Gasser RB 2000.** Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the *Contracaecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae). *Parasitology Research* 86(8):677–683.
Doi: [10.1007/PL00008551](https://doi.org/10.1007/PL00008551)
- Zhu X, Gasser RB, Podolska M & Chilton NB 1998.** Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *International Journal of Parasitology* 28(12):1911–1921.
Doi: [10.1016/S0020-7519\(98\)00150-7](https://doi.org/10.1016/S0020-7519(98)00150-7)

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