

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Nyman, Tommi; Papadopoulou, Elena; Ylinen, Eeva; Wutke, Saskia; Michell, Craig T.; Sromek, Ludmila; Sinisalo, Tuula; Andrievskaya, Elena; Alexeev, Vyacheslav; Kunnasranta, Mervi

Title: DNA barcoding reveals different cestode helminth species in northern European marine and freshwater ringed seals

Year: 2021

Version: Published version

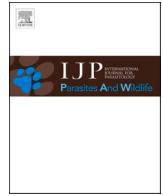
Copyright: © 2021 Published by Elsevier Ltd on behalf of Australian Society for Parasitology.

Rights: CC BY-NC-ND 4.0

Rights url: <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Please cite the original version:

Nyman, T., Papadopoulou, E., Ylinen, E., Wutke, S., Michell, C. T., Sromek, L., Sinisalo, T., Andrievskaya, E., Alexeev, V., & Kunnasranta, M. (2021). DNA barcoding reveals different cestode helminth species in northern European marine and freshwater ringed seals. *International Journal for Parasitology : Parasites and Wildlife*, 15, 255-261.
<https://doi.org/10.1016/j.ijppaw.2021.06.004>



DNA barcoding reveals different cestode helminth species in northern European marine and freshwater ringed seals

Tommi Nyman^{a,*}, Elena Papadopoulou^b, Eeva Ylinen^b, Saskia Wutke^b, Craig T. Michell^b, Ludmila Sromek^c, Tuula Sinisalo^d, Elena Andrievskaya^e, Vyacheslav Alexeev^e, Mervi Kunnasranta^{f,b}

^a Department of Ecosystems in the Barents Region, Norwegian Institute of Bioeconomy Research, Svanvik, Norway

^b Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

^c Department of Marine Ecosystems Functioning, Institute of Oceanography, University of Gdansk, Gdynia, Poland

^d Department of Biological and Environmental Sciences, University of Jyväskylä, Jyväskylä, Finland

^e The Baltic Ringed Seal Foundation, St. Petersburg, Russia

^f Natural Resources Institute Finland, Joensuu, Finland

ARTICLE INFO

Keywords:

COI barcoding
Pusa hispida
 Cestoda
 Freshwater seals
Schistocephalus solidus
Ligula intestinalis

ABSTRACT

Three subspecies of the ringed seal (*Pusa hispida*) are found in northeastern Europe: *P. h. botnica* in the Baltic Sea, *P. h. saimensis* in Lake Saimaa in Finland, and *P. h. ladogensis* in Lake Ladoga in Russia. We investigated the poorly-known cestode helminth communities of these closely related but ecologically divergent subspecies using COI barcode data. Our results show that, while cestodes from the Baltic Sea represent *Schistocephalus solidus*, all worms from the two lakes are identified as *Ligula intestinalis*, a species that has previously not been reported from seals. The observed shift in cestode communities appears to be driven by differential availability of intermediate fish host species in marine vs. freshwater environments. Both observed cestode species normally infect fish-eating birds, so further work is required to elucidate the health and conservation implications of cestode infections in European ringed seals, whether *L. intestinalis* occurs also in marine ringed seals, and whether the species is able to reproduce in seal hosts. In addition, a deep barcode divergence found within *S. solidus* suggests the presence of cryptic diversity under this species name.

1. Introduction

From a parasitological perspective, the three subspecies of the ringed seal (*Pusa hispida* (Schreber, 1775)) found in northeastern Europe form an interesting study system: the Baltic ringed seal (*P. h. botnica*) inhabits the northern and eastern parts of the Baltic Sea, while endemic freshwater subspecies are found in Lake Saimaa (*P. h. saimensis*) in Finland and in Lake Ladoga (*P. h. ladogensis*) in Russia (Fig. 1A). The two landlocked subspecies owe their origin to isostatic land uplift (bedrock rebound), which separated populations of ringed seals into newly-formed lakes after the northward retreat and eventual disappearance of the Scandinavian ice sheet at the end of the Pleistocene (Saarnisto, 2011). The Saimaa ringed seal most likely became isolated already around 10,000 years ago, while Lake Ladoga and, hence, its seal population, are a few thousand years younger (Kunnasranta et al., 2021;

Nyman et al., 2014). All three subspecies experienced dramatic human-caused population declines during the last 150 years. The Saimaa ringed seal nearly went extinct in the 1980s, when the population reached a low of an estimated 150 individuals (Kunnasranta et al., 2021). Since then, a slow recovery has led to the current population of slightly over 400 seals, but the subspecies is still classified as endangered (Kunnasranta et al., 2021). Although the number of Ladoga and Baltic ringed seals were likewise substantially reduced, their population sizes remained in the thousands through the 20th century (Sundqvist et al., 2012; Trukhanova, 2013). The different origins and demographic histories of the three subspecies are reflected in their genetic makeup, so that the Saimaa ringed seal possesses far less genetic diversity than the two other subspecies (Nyman et al., 2014; Palo et al., 2003; Peart et al., 2020; Valtonen et al., 2012).

The existence of three closely related ringed seal subspecies with

* Corresponding author. Department of Ecosystems in the Barents Region, Svanhovd Research Station, Norwegian Institute of Bioeconomy Research NO-9925, Svanvik, Norway.

E-mail address: Tommi.Nyman@nibio.no (T. Nyman).

<https://doi.org/10.1016/j.ijppaw.2021.06.004>

Received 11 March 2021; Received in revised form 14 June 2021; Accepted 21 June 2021

Available online 24 June 2021

2213-2244/© 2021 Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

widely divergent habitats, population sizes, demographic trajectories, and genetic diversities presents many opportunities for research on the ecology and evolution of host–parasite relationships and on abiotic and biotic factors that influence the composition of parasite communities. Parasitological research focussing on European ringed seals also has clear practical implications for health monitoring and conservation of the populations; in particular, there is a need to understand whether the

low genetic diversity of the endangered Saimaa ringed seal exposes this unique subspecies to threats posed by emerging parasites and pathogens (Kunnasranta et al., 2021; McCallum and Dobson, 1995; Stringer and Linklater, 2014). Furthermore, given that the seals have diverged into three morphologically and genetically distinct subspecies, it is quite possible that also their specialist parasites constitute endangered taxonomic units worthy of protection (Carlson et al., 2020; Dougherty et al.,

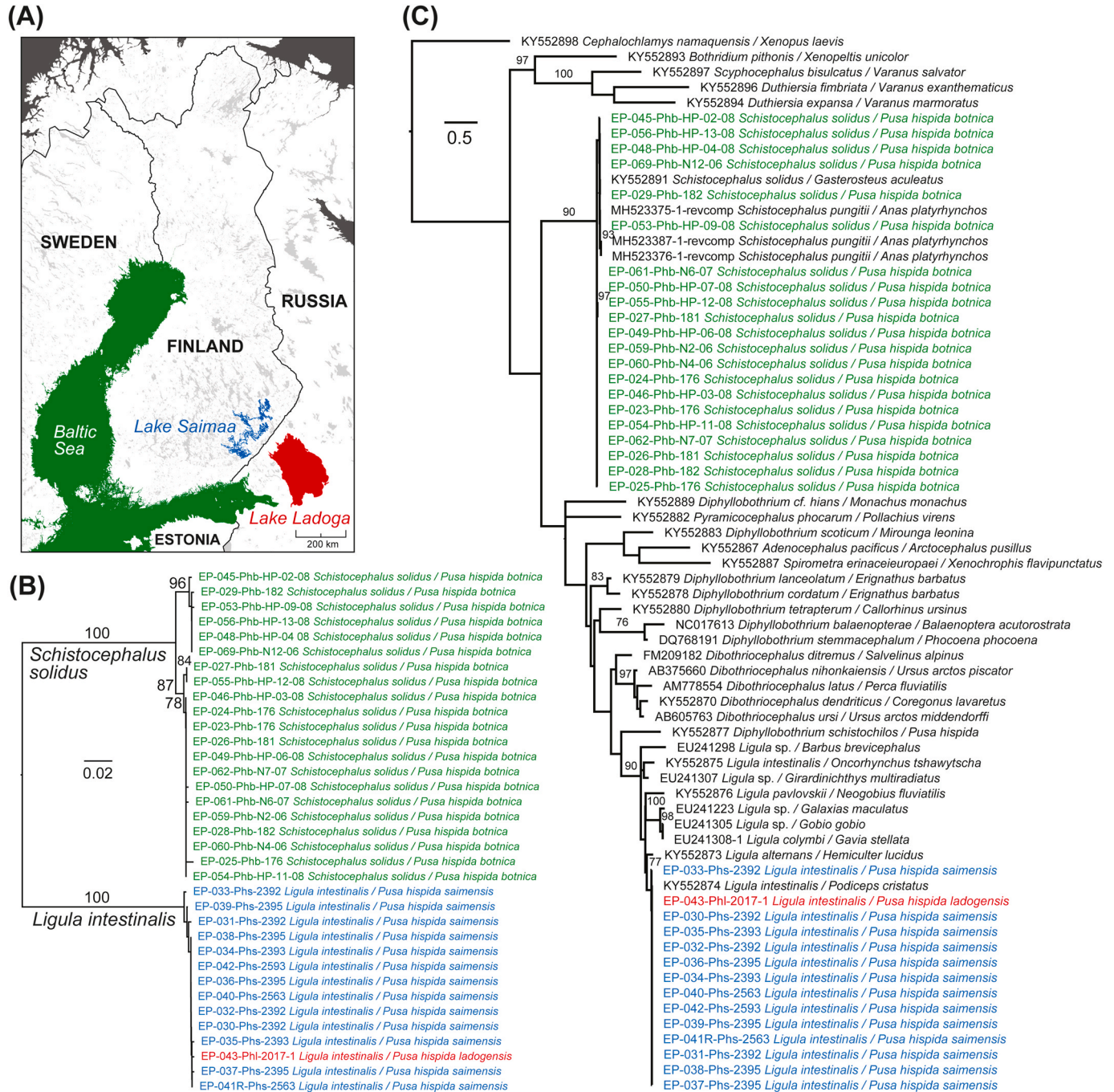


Fig. 1. (A) Geographic distributions of the three northern European ringed seal subspecies from which cestodes were collected for COI barcoding: Baltic ringed seal (green), Saimaa ringed seal (blue), and Ladoga ringed seal (red). (B) Midpoint-rooted neighbor-joining tree based on K2P distances among COI barcode sequences of 35 cestode individuals collected from the three focal ringed seal subspecies. Individuals are colored according to host subspecies, numbers above or next to branches are bootstrap support values based on 500 resamplings of the data matrix (only values > 70% shown). Cestode species names indicated under the main branches are based on barcode similarity to reference sequences in GenBank. (C) Maximum-likelihood tree based on a 562-bp alignment of the barcode sequences of the focal cestodes and 34 diphylobothriid reference taxa obtained from GenBank. Numbers above branches are bootstrap support values based on 100 resamplings of the data (only values > 70% shown). In both trees, individual names include the voucher code or GenBank accession number, seal subspecies abbreviation with seal individual code, barcode-based cestode species name, and name of the host (sub)species from which the cestode specimen was collected. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2016).

Like other subspecies of the circumpolarly distributed ringed seal (Felix, 2013; Johansen et al., 2010; Kelly et al., 2010; Morávková, 2016; Popov et al., 1980; Sinisalo, 2007; Vlasman and Campbell, 2004; Walden et al., 2020), Baltic, Saimaa, and Ladoga ringed seals are known to host a diverse complement of parasites. The collective parasite community of the three European subspecies consists of the ectoparasitic seal louse *Echinophthirius horridus* (von Olfers, 1816) (Leidenberger et al., 2007) and a diverse set of helminth worms comprising acanthocephalans (Leidenberger et al., 2020; Sinisalo et al., 2003), nematodes (Kelly et al., 2010; Westerling et al., 2005), and cestodes (Haukisalmi, 2015). Based on current knowledge, the Saimaa ringed seal lacks at least three endoparasites found in the more numerous subspecies: the seal heartworm *Acanthocheilonema spirocauda* (Leidy, 1858) (Nematoda: Onchocercidae) as well as two acanthocephalan species, *Corynosoma sermerae* (Forssell, 1904) and *C. strunosum* (Rudolphi, 1802) (Kunnasranta et al., 2021; Sinisalo et al., 2003; Westerling et al., 2005).

While numerous cestode species have been observed in the intestines of ringed seals, they tend to have low levels of both prevalence and intensity (Bergman, 2007; Geraci and St. Aubin, 1987; Johansen et al., 2010; Kelly et al., 2010; Measures and Gosselin, 1994; Walden et al., 2020). The presence of cestodes in northern European ringed seals is thought to result mainly from accidental infections by species that normally mature and reproduce in fish-feeding birds, but which have complex life cycles involving crustaceans and fish as intermediate hosts (Chubb et al., 1995; Haukisalmi, 2015; Sinisalo et al., 2006). Nevertheless, locally high prevalences and intensities of *Schistocephalus solidus* (Müller, 1776) cestodes have been found in Baltic ringed seals (Delyamure et al., 1980; Sinisalo et al., 2006) and, depending on the season, up to 11% of the individuals are able to reproduce in seals (Chubb et al., 1995). Seal cestode communities have overall been studied little, and usually alongside investigations of other intestinal helminths. In addition to *S. solidus*, unidentified diphylobothriids have been found in Baltic ringed seals (Bergman, 2007; Kelly et al., 2010; Sinisalo, 2007). For the Saimaa ringed seal, Sinisalo et al. (2003) and Sinisalo (2007) reported *Diphylobothrium ditretum* (Creplin, 1825) (misspelled as 'ditretum') and *Schistocephalus* sp. plerocercoids, and Haukisalmi (2015) reported the presence of the fish parasite *Triaenophorus nodulosus* (Pallas, 1781) in his checklist of tapeworms in Finnish vertebrates. Parasitism in Ladoga ringed seals has not been monitored systematically after the study by Delyamure et al. (1980), who found two unidentified diphylobothriid specimens in one out of 18 seals that they dissected.

The purpose of the present study was to clarify the species composition of cestode helminths of northern European ringed seals based on intestinal parasite material accumulated during long-term seal health monitoring and conservation programs. Because morphological identification of cestode worms is difficult and often uncertain (Hernández-Orts et al., 2015; Scholz et al., 2019; Waeschenbach et al., 2017), we sequenced the COI barcode region from cestodes collected from Baltic, Saimaa, and Ladoga ringed seals, and then compared the sequences to each other as well as to reference sequences obtained from public databases. Our results reveal that, while the cestodes from the Baltic Sea are as expected *Schistocephalus solidus*, all worms originating from the two lakes instead represent *Ligula intestinalis* (Linnaeus, 1758), a bird-infecting cestode species that has previously not been reported from seals. Our molecular data also indicate a deep barcode divergence within *S. solidus*, suggesting the presence of two species under this name. Although further sampling is needed for full characterization of cestode communities in ringed seals, our results suggest that cestode occurrence patterns are predominantly shaped by the differential availability of intermediate hosts in marine vs. freshwater environments.

2. Material and methods

2.1. Sample collection

The 35 cestode helminth worms sequenced in this study originated from the digestive organs (stomach, small and large intestine) of 17 Baltic ringed seals, five Saimaa ringed seals and one Ladoga ringed seal (Table 1). The number of barcoded specimens per seal ranged from one to four. All seals were found dead (Saimaa and Ladoga) or sampled for research purposes by shooting (Baltic) as part of long-term seal health monitoring programs of the University of Eastern Finland, Natural Resources Institute Finland, and Metsähallitus in Finland, and the Baltic Ringed Seal Foundation in Russia (research and sampling permits MMM 234/400/2008 and VARELY/3480/2016). Cestodes were collected during necropsies regularly conducted by the Finnish Food Authority and the Baltic Ringed Seal Foundation (Kunnasranta et al., 2021; Sinisalo et al., 2003), and were stored in 70–99.5% ethanol or water at –20 °C.

2.2. DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from circa 1.5 * 0.5 cm pieces of individual cestodes using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. We sequenced a 562-bp portion of the cytochrome c oxidase 1 (COI) barcoding gene by using the PCR amplification primers PBI-cox1F_PCR (5'-CAT TTT GCT GCC GGT CAR CAY ATG TTY TGR TTT TTT GG-3') and PBI-cox1R_PCR (5'-CCT TTG TCG ATA CTG CCA AAR TAA TGC ATD GGR AA-3'), and the sequencing primers PBI-cox1F_seq (5'-CAT TTT GCT GCC GGT CA-3') and PBI-cox1R_seq (5'-TAA TGC ATD GGR AAA AAA C-3') (Scholz et al., 2013). One µl of DNA extract and 0.33 µM of each primer were used in each 30-µl PCR reaction performed using the REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich), and the PCR cycling conditions were as follows: denaturation for 2 min at 94 °C, followed by 40 cycles of 30 s denaturation at 94 °C, 30 s annealing at 50 °C, and 1 min extension at 72 °C; followed by a final 10 min extension at 72 °C. PCR products were checked on 1.5% agarose gels stained with ethidium bromide before purification with the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Purified products were sequenced at Macrogen Europe, and the sequences were assembled and edited in Sequencher v.4.9 (GeneCodes Corporation).

2.3. Reference sequences and alignment

To place our sequences (Supplementary Data 1) in a broader phylogenetic context, we downloaded 34 COI sequences representing 30 diphylobothriidean species in twelve genera and three families from GenBank. The main part of these reference sequences were obtained by selecting one sequence for each species listed in Table 1 of Waeschenbach et al. (2017), but we also added three sequences for *Schistocephalus pungitii* Dubinina, 1959 (MH523375, MH523376, and MH523387) and one for *Ligula colymbi* Zeder, 1803 (EU241308). The sequences were aligned using MAFFT (Katoh and Standley, 2013) on the EMBL–EBI server (Madeira et al., 2019), resulting in a 562-bp matrix with 69 barcode region sequences (Supplementary Data 1).

Finally, we used the automated PhyloTA-R pipeline (Bennett et al., 2018) to retrieve full-length COI sequences (and their orthologues through all-vs-all BLAST) of the family Diphylobothriidae from GenBank, and aligned the sequences using MAFFT. The initial search resulted in 1489 COI sequences, of which 598 were left after removing duplicates. After deleting sequences that did not overlap with ours by at least 300 bp, we were left with a 1589-bp alignment with 353 COI sequences (Supplementary Data 1).

Table 1

Collection data for the 35 cestode specimens sequenced in this study and their seal host individuals. Cestode species were determined based on COI barcode similarity to reference sequences in GenBank. In the Host organ column, numbers in parentheses refer to equal-sized sections from the beginning towards the end of the small (ten sections) and large (four sections) intestine. Detailed intestinal sections are given only for those cestode specimens for which location data were recorded during host necropsies, and age estimates (in full years) only for those host individuals that had been aged based on counts of cementum layers in their lower canine teeth (see [Kunnasranta et al., 2021](#)).

Cestode species (individual code)	Host subspecies	Host individual code	Host organ	Host sex	Host age	Location	Collection date
<i>Schistocephalus solidus</i>							
EP_045	<i>P. h. botnica</i>	Phb_HP_02_08	Intestine	Female	Juv (1)	Finland: Baltic Sea, Tiurasen Krunni	30-May-2008
EP_046	<i>P. h. botnica</i>	Phb_HP_03_08	Intestine	Male	Ad (13)	Finland: Baltic Sea, Tiurasen Krunni	30-May-2008
EP_048	<i>P. h. botnica</i>	Phb_HP_04_08	Stomach	Female	Juv (1)	Finland: Baltic Sea, Tiurasen Krunni	30-May-2008
EP_049	<i>P. h. botnica</i>	Phb_HP_06_08	Intestine	Female	Juv (1)	Finland: Baltic Sea, Simon Möyly	01-Jun-2008
EP_050	<i>P. h. botnica</i>	Phb_HP_07_08	Intestine	Male	Juv (1)	Finland: Baltic Sea, Simon Möyly	01-Jun-2008
EP_053	<i>P. h. botnica</i>	Phb_HP_09_08	Intestine	Male	Ad (25)	Finland: Baltic Sea, Maakrunni Pohjoiskarikko	05-Jun-2008
EP_054	<i>P. h. botnica</i>	Phb_HP_11_08	Intestine	Male	Juv (2)	Finland: Baltic Sea, Kraasukka	07-Jun-2008
EP_055	<i>P. h. botnica</i>	Phb_HP_12_08	Intestine	Male	Ad (15)	Finland: Baltic Sea, Simon Möyly	09-Jun-2008
EP_056	<i>P. h. botnica</i>	Phb_HP_13_08	Intestine	Female	Ad (8)	Finland: Baltic Sea, Tiurasen Krunni	09-Jun-2008
EP_059	<i>P. h. botnica</i>	Phb_N2_06	Intestine	Female	Ad (7)	Finland: Baltic Sea, Bothnian Bay	20-Apr-2006
EP_060	<i>P. h. botnica</i>	Phb_N4_06	Intestine	Female	Ad (6)	Finland: Baltic Sea, Bothnian Bay	21-Apr-2006
EP_061	<i>P. h. botnica</i>	Phb_N6_07	Intestine	Female	Ad (16)	Finland: Baltic Sea, Bothnian Bay	23-Apr-2007
EP_062	<i>P. h. botnica</i>	Phb_N7_07	Intestine	Male	Ad (7)	Finland: Baltic Sea, Bothnian Bay	23-Apr-2007
EP_069	<i>P. h. botnica</i>	Phb_N12_06	Intestine	Female	Ad (13)	Finland: Baltic Sea, Bothnian Bay	23-Apr-2006
EP_023	<i>P. h. botnica</i>	Phb_176	Large intestine (1)	Male	Juv (1)	Finland: Baltic Sea, Bothnian Bay	20-Apr-2016
EP_024	<i>P. h. botnica</i>	Phb_176	Small intestine (10)	Male	Juv (1)	Finland: Baltic Sea, Bothnian Bay	20-Apr-2016
EP_025	<i>P. h. botnica</i>	Phb_176	Small intestine (9)	Male	Juv (1)	Finland: Baltic Sea, Bothnian Bay	20-Apr-2016
EP_026	<i>P. h. botnica</i>	Phb_181	Small intestine (10)	Female	Juv (2)	Finland: Baltic Sea, Bothnian Bay	09-May-2016
EP_027	<i>P. h. botnica</i>	Phb_181	Large intestine (1)	Female	Juv (2)	Finland: Baltic Sea, Bothnian Bay	09-May-2016
EP_028	<i>P. h. botnica</i>	Phb_182	Large intestine (1)	Female	Juv (1)	Finland: Baltic Sea, Bothnian Bay	09-May-2016
EP_029	<i>P. h. botnica</i>	Phb_182	Large intestine (2)	Female	Juv (1)	Finland: Baltic Sea, Bothnian Bay	09-May-2016
<i>Ligula intestinalis</i>							
EP_030	<i>P. h. saimensis</i>	Phs_2392	Intestine	Female	Juv (0)	Finland: Lake Saimaa, Haukivesi	05-Aug-2005
EP_031	<i>P. h. saimensis</i>	Phs_2392	Intestine	Female	Juv (0)	Finland: Lake Saimaa, Haukivesi	05-Aug-2005
EP_032	<i>P. h. saimensis</i>	Phs_2392	Intestine	Female	Juv (0)	Finland: Lake Saimaa, Haukivesi	05-Aug-2005
EP_033	<i>P. h. saimensis</i>	Phs_2392	Intestine	Female	Juv (0)	Finland: Lake Saimaa, Haukivesi	05-Aug-2005
EP_034	<i>P. h. saimensis</i>	Phs_2393	Intestine	Male	Juv (0)	Finland: Lake Saimaa, Joutenvesi	14-Aug-2005
EP_035	<i>P. h. saimensis</i>	Phs_2393	Intestine	Male	Juv (0)	Finland: Lake Saimaa, Joutenvesi	14-Aug-2005
EP_036	<i>P. h. saimensis</i>	Phs_2395	Intestine	Female	Juv (1)	Finland: Lake Saimaa, Tolvanselka	01-Jan-2006
EP_037	<i>P. h. saimensis</i>	Phs_2395	Intestine	Female	Juv (1)	Finland: Lake Saimaa, Tolvanselka	01-Jan-2006
EP_038	<i>P. h. saimensis</i>	Phs_2395	Intestine	Female	Juv (1)	Finland: Lake Saimaa, Tolvanselka	01-Jan-2006
EP_039	<i>P. h. saimensis</i>	Phs_2395	Intestine	Female	Juv (1)	Finland: Lake Saimaa, Tolvanselka	01-Jan-2006
EP_040	<i>P. h. saimensis</i>	Phs_2563	Intestine	Female	Juv (0)	Finland: Lake Saimaa, Pihlajavesi	14-Jan-2013
EP_041 R	<i>P. h. saimensis</i>	Phs_2563	Small intestine (10)	Female	Juv (0)	Finland: Lake Saimaa, Pihlajavesi	14-Jan-2013
EP_042	<i>P. h. saimensis</i>	Phs_2593	Intestine	Male	Juv (1)	Finland: Lake Saimaa, Haukivesi	09-Feb-2014
EP_043	<i>P. h. ladogensis</i>	Phl_2017_1	Intestine	Male	Juv	Russia: Lake Ladoga, Sortavala	Aug-2017

2.4. Phylogeny reconstruction and species identification

To obtain an initial overview of sequence variation in our COI barcode dataset, we constructed a midpoint-rooted neighbor-joining tree for our own 35 ringed seal cestode sequences based on Kimura 2-parameter distances and pairwise deletion in Mega X (Kumar et al., 2018). Clade support was estimated based on 500 bootstrap resamplings of the data matrix. Based on the tree, we selected groups of barcode sequences that were identified to species by querying GenBank using the Basic Local Alignment Search Tool (BLAST) (Johnson et al., 2008). Searches implementing the sequence-based identification engine of the Barcode of Life Data System database (Ratnasingham and Hebert, 2007) did not produce hits to cestode species.

The 69- and 353-taxon datasets including reference sequences from GenBank were analyzed in a maximum-likelihood framework in RAxML BlackBox (Stamatakis, 2014) on the CIPRES server (Miller et al., 2010), implementing a GTR + G model of substitution partitioned according to codon positions 1 + 2 vs. 3, and with 100 bootstrap replicates to estimate clade support. The trees were rooted according to the four-gene phylogeny of Waeschenbach et al. (2017).

3. Results and discussion

Neighbor-joining clustering of the sequence data based on Kimura 2-parameter distances grouped our ringed seal cestode COI barcode sequences into two large clusters that were separated by an average K2P distance of 0.24 (Fig. 1B). BLAST searches of GenBank sequences revealed that the larger of the clusters represented *Schistocephalus solidus* or *S. pungitii* with >99% sequence identity to the closest reference sequences, while sequences of the smaller cluster produced hits to *Ligula intestinalis* barcodes with an >99% identity. These results were confirmed by ML analyses in which we combined our data with representative barcode sequences from the order Diphyllbothriidea (Fig. 1C) and all available COI sequences from the family Diphyllbothriidae (Supplementary Fig. S1). What is more, all 21 *S. solidus/pungitii* individuals originated from 17 Baltic ringed seals, while the 14 *L. intestinalis* worms originated from five Saimaa and one Ladoga ringed seal. The results therefore reveal clearly contrasting cestode communities in the marine ringed seals of the Baltic Sea vs. the two endemic freshwater subspecies inhabiting lakes Saimaa and Ladoga (Fig. 1A).

Our *L. intestinalis* barcodes formed a very tight clade (Supplementary Fig. S1) with reference specimens predominantly collected from cyprinid fish and fish-eating birds (mainly from Bouzid et al. (2008) and Waeschenbach et al. (2017)). Our finding of *L. intestinalis* in the landlocked seal populations was unexpected, although the apparently closely related *L. colymbi* (Fig. 1C and Supplementary Fig. S1; see also Bouzid et al. (2008)) has been found in the Caspian seal (*Pusa caspica* (Gmelin)) (Kalmykov, 2016). This raises the question of whether *L. intestinalis* has been overlooked or misidentified in the aforementioned morphology-based surveys (Delyamure et al., 1980; Haukisalmi, 2015; Sinisalo, 2007; Sinisalo et al., 2003) of helminth communities of landlocked seals. The possibility is realistic, because cestodes are notoriously difficult to identify based on morphology (Waeschenbach et al., 2017), and many authors (e.g., Haukisalmi, 2015) have noted that published records and cestode host databases may contain considerable amounts of errors; identification errors and unstable taxonomy are also evidenced by the non-monophyly of many diphyllbothriid species across our 353-tip reference phylogeny (Supplementary Fig. S1). To make things worse, individuals sampled from seal intestines are often fragmented or in poor condition due to partial degradation in dead hosts (Hernández-Orts et al., 2015). Molecular-genetic methods provide reliable tools for identification of such samples, but the deep splits within the phylum presents challenges for designing universally functional PCR primers (Vanhove et al., 2013). This is exemplified by our own experiences: because our initial expectation was that all our samples are *S. solidus*, we first tried the *Schistocephalus*-specific COI primers of

Nishimura et al. (2011) and Sprehn et al. (2015), but quickly found out that they did not amplify any samples from Lake Saimaa and Lake Ladoga. Even the taxonomically widely applicable ‘Dice’ primers of Van Steenkiste et al. (2015) produced variable results, so our final protocol relied on the ‘PBI-cox1’ primers of Scholz et al. (2013).

Our results also reveal deep and well-supported barcode divergence within the *Schistocephalus* clade, so that specimens are grouped into two clusters consisting of six and 15 individuals, respectively (Fig. 1B). The mean K2P distance between individuals belonging to the same cluster is 0.002. By contrast, pairwise distances between individuals belonging to different clusters range from 0.018 to 0.025, with a mean of 0.0198. Mean among-group distances are therefore close to 0.02, which is applicable as a threshold for species delimitation in many animal taxa (Hebert et al., 2003). Individuals of the smaller cluster are grouped with two *S. solidus* and three *S. pungitii* reference sequences obtained from GenBank (Fig. 1C, Supplementary Fig. S1). However, the *S. solidus* reference sequence KY552891 (Waeschenbach et al., 2017) is derived from a specimen collected from threespine stickleback (*Gasterosteus aculeatus* L.), which is considered the only host of the plerocercoid stage of *S. solidus* (Henrich and Kalbe, 2016; Nishimura et al., 2011). Therefore, until the genetic properties of these two *Schistocephalus* species are characterized in more detail, we tentatively consider this identification more reliable than *S. pungitii*, which is based on reference individuals collected from mallards (*Anas platyrhynchos* L.) (Prüter et al., 2018). Our larger *S. solidus* cluster did not produce close hits to previously-published sequences, but we note that Chubb et al. (1995) found bimodal distributions of segment numbers within *S. solidus* (see also Chubb et al., 2006), which suggests the presence of more than one species under the name. Nishimura et al. (2011) demonstrated a deep sequence divergence between *S. solidus* specimens collected from North America and Europe, but the existence of additional undetected European species would not be surprising considering that new *Schistocephalus* species have been described relatively recently from northern Finland (Chubb et al., 2006).

The shift in cestode communities between marine vs. freshwater ringed seals appears to be driven mainly by the differential availability of intermediate hosts in the different habitats. This seems to explain especially the absence of *S. solidus* from Lake Saimaa, as the distribution of *G. aculeatus* does not extend to the lake (Natural Resources Institute Finland, 2021). However, threespine stickleback is present in Lake Ladoga (Kudersky et al., 1996), and *Schistocephalus* species are found in fish (Rumyantsev and Ieshko, 1997) and terns (Lebedeva et al., 2020) in the region. Therefore, with the current sampling we cannot exclude the possibility that *S. solidus* would occasionally be present in Ladoga ringed seals as well. *L. intestinalis* is more flexible with respect to intermediate fish hosts, although it predominantly circulates via cyprinids (Haukisalmi, 2015; Orr, 1967). Cyprinids are common in both of the focal lakes, and *L. intestinalis* has been recorded from fish (Rumyantsev and Ieshko, 1997) and cormorants (*Phalacrocorax carbo* (L.)) feeding in Lake Ladoga (Yakovleva et al., 2020). However, although cyprinid fish are near-exclusively confined to freshwater habitats, many cyprinid species thrive in the brackish-water Baltic Sea, where they are also infected by *L. intestinalis* plerocercoids (Glazunova and Polunina, 2009). Therefore, the possibility of finding the species in Baltic ringed seals still remains.

In conclusion, our results demonstrate that different cestode species infect northern European marine vs. landlocked ringed seals. We suggest that the shift is driven by habitat-specific availability and abundance of intermediate hosts needed for completing the complex life cycles of *S. solidus* and *L. intestinalis* (threespine stickleback and cyprinids, respectively). However, more thorough studies of the parasite communities of especially Ladoga ringed seals are needed for assessment of the differences and possible underlying causes. Further work is also required for elucidating the possible presence of cryptic diversity within *S. solidus*, as well as the ecological importance and conservation implications of cestode infections in endangered landlocked seals. As shown by Chubb et al. (1995), *S. solidus* prevalence and abundance can be high

in the Baltic Sea, and at least some of the individuals are able to reproduce in seals. Whether this is the case also for *L. intestinalis* in the landlocked subspecies remains to be studied.

Data availability

COI barcode sequences of the cestodes analyzed here are available in GenBank (accession numbers MZ359917–MZ359951). The three alignments and resultant phylogenetic trees are included in a Nexus-formatted text file as Supplementary Data 1.

Declaration of competing interest

All authors declare no conflicts of interest.

Acknowledgments

We wish to thank especially researchers and students of the UEF's Saimaa ringed seal research group, Metsähallitus, and the Finnish Food Agency, who have collected intestinal parasites of ringed seals during their conservation and monitoring activities. We also thank Evgeny Ieshko for information on fish and seal parasites in Lake Ladoga, Marja Isomursu for helpful discussions on the occurrence of seal parasites in the Baltic Sea and Lake Saimaa, and Timothy Littlewood for sharing experiences on cestode barcoding primers. Funding for laboratory work and reagents was provided by the Raija and Ossi Tuuliainen Foundation, the Betty Väänänen Foundation, Societas Pro Fauna et Flora Fennica, and the Nestori Foundation. The Norwegian Biodiversity Information Facility (Artsdatabanken) provided partial funding for TN (Project 27-19), the Polish National Science Centre (grant no. 2019/32/C/NZ8/00335) for LS, and the EU's Erasmus+ programme kindly supported the research visitor project by EP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2021.06.004>.

References

- Bennett, D.J., Hettling, H., Silvestro, D., Zizka, A., Bacon, C.D., Faurby, S., Vos, R.A., Antonelli, A., 2018. PhylotaR: an automated pipeline for retrieving orthologous DNA sequences from GenBank in R. *Life* 8, e20. <https://doi.org/10.3390/life8020020>.
- Bergman, A., 2007. Pathological Changes in Seals in Swedish Waters: the Relation to Environmental Pollution Tendencies during a 25-year Period. PhD Thesis. Swedish University of Agricultural Sciences.
- Bouzid, W., Štefka, J., Hypša, V., Lek, S., Scholz, T., Legal, L., Hassine, O.K. Ben, Loot, G., 2008. Geography and host specificity: two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphylobothriidae). *Int. J. Parasitol.* 38, 1465–1479. <https://doi.org/10.1016/j.ijpara.2008.03.008>.
- Carlson, C.J., Hopkins, S., Bell, K.C., Doña, J., Godfrey, S.S., Kwak, M.L., Lafferty, K.D., Moir, M.L., Speer, K.A., Strona, G., Torchin, M., Wood, C.L., 2020. A global parasite conservation plan. *Biol. Conserv.* 250, e108596 <https://doi.org/10.1016/j.biocon.2020.108596>.
- Chubb, J.C., Seppälä, T., Lüscher, A., Milinski, M., Valtonen, E.T., 2006. *Schistocephalus cotti* n. sp. (Cestoda: Pseudophyllidea) plerocercoids from bullheads *Cottus gobio* L. in an Arctic river in Finland, with a key to the plerocercoids of the Palaearctic species of the genus. *Syst. Parasitol.* 65, 161–170. <https://doi.org/10.1007/s11230-006-9047-5>.
- Chubb, J.C., Valtonen, E.T., McGeorge, J., Helle, E., 1995. Characterisation of the external features of *Schistocephalus solidus* (Müller, 1776) (Cestoda) from different geographical regions and an assessment of the status of the Baltic ringed seal *Phoca hispida botnica* (Gmelin) as a definitive host. *Syst. Parasitol.* 32, 113–123. <https://doi.org/10.1007/BF00009510>.
- Delyamure, S.L., Popov, V.N., Trashchenkov, A.N., 1980. A study of the helminth fauna of seals in the Baltic Sea and Lake Ladoga. *Nauchnye Dokl. Vyssh. Shkoly Biol. Nauk.* 7, 43–45.
- Dougherty, E.R., Carlson, C.J., Bueno, V.M., Burgio, K.R., Cizauskas, C.A., Clements, C.F., Seidel, D.P., Harris, N.C., 2016. Paradigms for parasite conservation. *Conserv. Biol.* 30, 724–733. <https://doi.org/10.1111/cobi.12634>.
- Felix, J.R., 2013. Reported Incidences of Parasitic Infections in Marine Mammals from 1892 to 1978. *Zea Books, Lincoln (Nebraska)*.
- Geraci, J.R., St Aubin, D.J., 1987. Effects of parasites on marine mammals. *Int. J. Parasitol.* 17, 407–414. [https://doi.org/10.1016/0020-7519\(87\)90116-0](https://doi.org/10.1016/0020-7519(87)90116-0).
- Glazunova, A.A., Polunina, Y.Y., 2009. Copepods as the first intermediate hosts of *Ligula intestinalis* L.: parasite of bream *Abramis brama* L. in the Vistula Lagoon of the Baltic Sea. *Int. Water Biol.* 2, 371–376. <https://doi.org/10.1134/S1995082909040129>.
- Haukialmi, V., 2015. Checklist of tapeworms (Platyhelminthes, Cestoda) of vertebrates in Finland. *ZooKeys* 533, 1–61. <https://doi.org/10.3897/zookeys.533.6538>.
- Hebert, P.D.N., Ratnasingham, S., DeWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B Biol. Sci.* 270, 96–99. <https://doi.org/10.1098/rsbl.2003.0025>.
- Henrich, T., Kalbe, M., 2016. The role of prezygotic isolation mechanisms in the divergence of two parasite species. *BMC Evol. Biol.* 16, e245 <https://doi.org/10.1186/s12862-016-0799-5>.
- Hernández-Orts, J.S., Scholz, T., Brabec, J., Kuzmina, T., Kuchta, R., 2015. High morphological plasticity and global geographical distribution of the Pacific broad tapeworm *Adenocephalus pacificus* (syn. *Diphylobothrium pacificum*): molecular and morphological survey. *Acta Trop.* 149, 168–178. <https://doi.org/10.1016/j.actatropica.2015.05.017>.
- Johansen, C.E., Lydersen, C., Aspholm, P.E., Haug, T., Kovacs, K.M., 2010. Helminth parasites in ringed seals (*Pusa hispida*) from Svalbard, Norway with special emphasis on nematodes: variation with age, sex, diet, and location of host. *J. Parasitol.* 96, 946–953. <https://doi.org/10.1645/GE-1685.1>.
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezuk, Y., McGinnis, S., Madden, T.L., 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res.* 36 <https://doi.org/10.1093/nar/gkn201>. W5–W9.
- Kalmykov, A.P., 2016. New definitive hosts of the ligulids (Ligulidae) in the delta of Volga. *Am. Sci. J.* 4, 117–120.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Kelly, B.P., Bengtson, J.L., Boveng, P.L., Cameron, M.F., Dahle, S.P., Jansen, J.K., Logerwell, E.A., Overland, J.E., Sabine, C.L., Waring, G.T., Wilder, J.M., 2010. Status review of the ringed seal (*Phoca hispida*). NOAA Tech. Memo. NMFS-AFSC-212, 1–265.
- Kudersky, L.K., Jurvelius, J., Kaukoranta, M., Tuunainen, P., Mäkinen, K., 1996. Fishery of Lake Ladoga – past, present and future. *Hydrobiologia* 322, 57–64.
- Kumar, S., Stecher, G., Li, M., Nnyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Kunnasranta, M., Niemi, M., Autila, M., Valtonen, M., Kammonen, J., Nyman, T., 2021. Sealed in a lake — biology and conservation of the endangered Saimaa ringed seal: a review. *Biol. Conserv.* 253, e108908 <https://doi.org/10.1016/j.biocon.2020.108908>.
- Lebedeva, D.I., Yakovleva, G.A., Artem'ev, A.V., 2020. Parasites of common (*Sterna hirundo*) and Arctic (*Sterna paradisaea*) terns (Charadriiformes, Laridae) in Karelia. *Biol. Bull.* 47, 747–752. <https://doi.org/10.1134/S1062359020070092>.
- Leidenberger, S., Boström, S., Wayland, M.T., 2020. Host records and geographical distribution of *Corynosoma magdalenae*, C. semerme and C. strumsum (Acanthocephala: Polymorphidae). *Biodivers. Data J.* 8, e50500 <https://doi.org/10.3897/BDJ.8.e50500>.
- Leidenberger, S., Harding, K., Härkönen, T., 2007. Phocid seals, seal lice and heartworms: a terrestrial host-parasite system conveyed to the marine environment. *Dis. Aquat. Org.* 77, 235–253. <https://doi.org/10.3354/dao01823>.
- Madeira, F., Park, Y.M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A.R.N., Potter, S.C., Finn, R.D., Lopez, R., 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47, W636–W641. <https://doi.org/10.1093/nar/gkz268>.
- McCallum, H., Dobson, A., 1995. Detecting disease and parasite threats to endangered species and ecosystems. *Trends Ecol. Evol.* 10, 190–194.
- Measures, L.N., Gosselin, J.F., 1994. Helminth parasites of ringed seal, *Phoca hispida*, from northern Quebec, Canada. *J. Helminthol. Soc. Washington* 61, 240–244.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. 14 Nov. 2010, pp. 1–8. New Orleans, LA.
- Morávková, V., 2016. Diversity and Geographical Distribution of Tapeworms of the Order Diphylobothriidea in Pinnipedia. B.Sc. Thesis, University of South Bohemia in České Budějovice.
- Natural Resources Institute Finland, 2021. Kalahavainnot (Fish Observations) [WWW Document]. URL <http://kalahavainnot.luke.fi/kartta>. (Accessed 23 February 2021).
- Nishimura, N., Heins, D.C., Andersen, R.O., Barber, I., Cresko, W.A., 2011. Distinct lineages of *Schistocephalus* parasites in threespine and ninespine stickleback hosts revealed by DNA sequence analysis. *PLoS One* 6, e22505. <https://doi.org/10.1371/journal.pone.0022505>.
- Nyman, T., Valtonen, M., Aspi, J., Ruokonen, M., Kunnasranta, M., Palo, J., 2014. Demographic histories and genetic diversities of Fennoscandian marine and landlocked ringed seal subspecies. *Ecol. Evol.* 4, 3420–3434.
- Orr, T.S.C., 1967. Distribution of the plerocercoid of *Ligula intestinalis*. *J. Zool.* 153, 91–97.
- Palo, J.U., Hyvärinen, H., Helle, E., Mäkinen, H.S., Väinölä, R., 2003. Postglacial loss of microsatellite variation in the landlocked Lake Saimaa ringed seal. *Conserv. Genet.* 4, 117–128.
- Peart, C.R., Tusso, S., Pophaly, S.D., Botero-Castro, F., Wu, C.C., Auriolles-Gambo, D., Baird, A.B., Bickham, J.W., Forcada, J., Galimberti, F., Gemmill, N.J., Hoffman, J.I., Kovacs, K.M., Kunnasranta, M., Lydersen, C., Nyman, T., de Oliveira, L.R., Orr, A.J., Sanvito, S., Valtonen, M., Shafer, A.B.A., Wolf, J.B.W., 2020. Determinants of genetic variation across eco-evolutionary scales in pinnipeds. *Nat. Ecol. Evol.* 4, 1095–1104. <https://doi.org/10.1038/s41559-020-1215-5>.

- Popov, V.N., Jurakhno, M.V., Skriabin, V.A., 1980. Helminth fauna of the okhotsk ringed seal from the Patience Bay and Laperouse strait. *Parasitologija* 14, 48–55.
- Prüter, H., Franz, M., Auls, S., Czirják, G., Greben, O., Greenwood, A.D., Lisitsyna, O., Syrota, Y., Sitko, J., Krone, O., 2018. Chronic lead intoxication decreases intestinal helminth species richness and infection intensity in mallards (*Anas platyrhynchos*). *Sci. Total Environ.* 644, 151–160. <https://doi.org/10.1016/j.scitotenv.2018.06.297>.
- Ratnasingham, S., Hebert, P.D.N., 2007. BOLD : the barcode of life data system. *Mol. Ecol. Notes* 7, 355–364. <https://doi.org/10.1111/j.1471-8286.2006.01678.x>. www.barcodinglife.org.
- Rumyantsev, E.A., Ieshko, E.P., 1997. Parazity ryb vodoemov Karelii: sistematičeskii Katalog (fish parasites in Karelia: systematic Catalogue). *Karel. Nauch. Tsentr, Ross. Akad. Nauk, Petrozavodsk*.
- Saarnisto, M., 2011. Challenging the early holocene isolation dating of the Saimaa and Ladoga ringed seals. In: Harjula, J., Helamaa, M., Haarala, J. (Eds.). *Taavitsainen Festschrift Committee, Turku*, pp. 28–39. *Times, Things and Places: 36 Essays for Jussi-Pekka Taavitsainen*. J.-P.
- Scholz, T., de Chambrier, A., Kuchta, R., Littlewood, D.T.J., Waeschenbach, A., 2013. *Macrobothriotaenia ficta* (Cestoda: Proteocephalidea), a parasite of sunbeam snake (*Xenopeltis unicolor*): example of convergent evolution. *Zootaxa* 3640, 485–499. <https://doi.org/10.11646/zootaxa.3640.3.12>.
- Scholz, T., Kuchta, R., Brabec, J., 2019. Broad tapeworms (Diphyllobothriidae), parasites of wildlife and humans: recent progress and future challenges. *Int. J. Parasitol.* 49, 359–369. <https://doi.org/10.1016/j.ijppaw.2019.02.001>.
- Sinisalo, T., 2007. Diet and Foraging of Ringed Seals in Relation to Helminth Parasite Assemblages. Ph.D. Thesis, University of Jyväskylä.
- Sinisalo, T., Kunnasranta, M., Valtonen, E.T., 2003. Intestinal helminths of a landlocked ringed seal (*Phoca hispida saimensis*) population in eastern Finland. *Parasitol. Res.* 91, 40–45. <https://doi.org/10.1007/s00436-003-0893-7>.
- Sinisalo, T., Valtonen, E.T., Helle, E., Jones, R.L., 2006. Combining stable isotope and intestinal parasite information to evaluate dietary differences between individual ringed seals (*Phoca hispida botnica*). *Can. J. Zool.* 84, 823–831. <https://doi.org/10.1139/z06-067>.
- Sprehn, C.G., Blum, M.J., Quinn, T.P., Heins, D.C., 2015. Landscape genetics of *Schistocephalus solidus* parasites in threespine stickleback (*Gasterosteus aculeatus*) from Alaska. *PLoS One* 10, e0122307. <https://doi.org/10.1371/journal.pone.0122307>.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
- Stringer, A.P., Linklater, W., 2014. Everything in moderation: principles of parasite control for wildlife conservation. *Bioscience* 64, 932–937. <https://doi.org/10.1093/biosci/biu135>.
- Sundqvist, L., Harkonen, T., Svensson, C.J., Harding, K.C., 2012. Linking climate trends to population dynamics in the Baltic ringed seal: impacts of historical and future winter temperatures. *Ambio* 41, 865–872. <https://doi.org/10.1007/s13280-012-0334-x>.
- Trukhanova, I.S., 2013. The Ladoga ringed seal (*Pusa hispida ladogensis*) under changing climatic conditions. *Russ. J. Theriol.* 12, 41–48.
- Valtonen, M., Palo, J.U., Ruokonen, M., Kunnasranta, M., Nyman, T., 2012. Spatial and temporal variation in genetic diversity of an endangered freshwater seal. *Conserv. Genet.* 13, 1231–1245. <https://doi.org/10.1007/s10592-012-0367-5>.
- Van Steenkiste, N., Locke, S.A., Castelin, M., Marcogliese, D.J., Abbott, C.L., 2015. New primers for DNA barcoding of digeneans and cestodes (Platyhelminthes). *Mol. Ecol. Resour.* 15, 945–952. <https://doi.org/10.1111/1755-0998.12358>.
- Vanhove, M.P.M., Tessens, B., Schoelincx, C., Jondelius, U., Littlewood, D.T.J., Artois, T., Huysse, T., 2013. Problematic barcoding in flatworms: a case-study on monogeneans and rhabdocoels (Platyhelminthes). *ZooKeys* 365, 355–379. <https://doi.org/10.3897/zookeys.365.5776>.
- Vlasman, K., Campbell, D., 2004. *Field Guide: Diseases and Parasites of Marine Mammals of the Eastern Arctic*. Canadian Cooperative Wildlife Health Centre, Guelph.
- Waeschenbach, A., Brabec, J., Scholz, T., Littlewood, D.T.J., Kuchta, R., 2017. The catholic taste of broad tapeworms – multiple routes to human infection. *Int. J. Parasitol.* 47, 831–843. <https://doi.org/10.1016/j.ijpara.2017.06.004>.
- Walden, H.S., Bryan, A.L., McIntosh, A., Tuomi, P., Hoover-Miller, A., Stimmelmayr, R., Quakenbush, L., 2020. Helminth fauna of ice seals in the Alaskan Bering and Chukchi seas, 2006–15. *J. Wildl. Dis.* 56, 863–872. <https://doi.org/10.7589/2019-09-228>.
- Westerling, B., Stenman, O., Rudbäck, E., 2005. Pathology of seals from the Finnish coastal waters, Lake Saimaa and Lake Ladoga. In: Helle, E., Stenman, O., Wikman, M. (Eds.), *Symposium on Biology and Management of Seals in the Baltic Area*. Finnish Game and Fisheries Research Institute, Helsinki, pp. 76–79.
- Yakovleva, G.A., Artem'ev, A.V., Lebedeva, D.I., 2020. Expansion of the great cormorant (*Phalacrocorax carbo* L. 1758) to the northwest of Russia as a possibility of the spread of parasites. *Russ. J. Biol. Invasions* 11, 92–96. <https://doi.org/10.1134/S2075111720010129>.