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Lecithaster (Lecithasteridae, Digenea) in the White Sea: an unnoticed guest from the Pacific?

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Research Paper

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Abstract

Morphological discrimination of species is problematic in many digenean taxa. Parasites of marine fish from the genus *Lecithaster* Lühe, 1901 are a good example of this. Our goal was to understand which species of *Lecithaster* infect fish in the White Sea, and reveal their life cycles. We collected specimens of maritae from nine fish species, analysed their morphology and sequenced 28S ribosomal DNA and internal transcribed spacer 2 (ITS2). Contrary to previous accounts, all of them belong to a single species, *Lecithaster salmonis* Yamaguti, 1934, which was previously only recorded from the Pacific. Morphologically, our maritae specimens were highly variable, sharing characters of *L. salmonis*, *Lecithaster confusus* Odhner, 1905 and *Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901. This variability did not correlate with the moderate differences in ITS2 among the specimens, and neither did the fish host species. Members of the subfamily Salmoninae appear to be the best suited definitive hosts, judging from the intensity rates. The intermediate hosts were also discovered: the first is *Cryptonatica affinis* (Gmelin, 1791) and the second are planktonic copepods. These life-cycle data from the White Sea are consistent with *L. salmonis* species identification and with the distribution of this species in the North Pacific. The geographical range of *L. salmonis* seems to be interrupted, and we discuss possible ways of *L. salmonis* expansion.

Introduction

Digeneans from the genus *Lecithaster* Lühe, 1901 (family Lecithasteridae Odhner, 1905, superfamily Hemiuroidea Looss, 1899) are globally distributed parasites of marine fish (Gibson, 2002; WoRMS, 2022a). *Lecithaster* comprises 31 valid species, and in many of them maritae (sexual adults) are quite similar, with only morphometric analysis helping to tell them apart (Srivastava, 1966; Martin & Multani, 1970; Orecchia et al., 1988; Besprozvannykh et al., 2017). Intensive molecular genetic studies have been performed for the *Lecithaster* species from the Far East recently (Besprozvannykh et al., 2017; Atopkin et al., 2020), but relevant sequencing data on this genus from the European waters are surprisingly few (Olson et al., 2003). Alongside molecules, characters that help with the species delineation are associated with the lifecycle pattern. Recently, several digenean species were shown to have the same morphology of marita, although the lifecycle stages from the intermediate hosts and the species range of these hosts differ (Gilardoni et al., 2020; Gonchar & Galaktionov, 2021).

In the present article, we question the specific identity of the *Lecithaster* found in White Sea fishes. Previously, *Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901 and *Lecithaster confusus* Odhner, 1905 had been recorded in this region (Shulman & Shulman-Albova, 1953; Mitenev & Karasev, 2005). However, these species use *Brachystomia eulimoides* (Hanley, 1844) and *Boonea trifida* (Totten, 1834) (Heterobranchia: Pyramidellidae) as their first intermediate hosts, respectively (Hunninen & Cable, 1943; Køie, 1989). These gastropods have never been found in the White Sea (Golikov, 1987; Kantor & Sysoev, 2006). However, lecithasterid sporocysts and cystophorous cercariae, *Cercaria saccocaudata* Tschubrik, 1966, have been found in another gastropod, *Cryptonatica affinis* (Gmelin, 1791) (Caenogastropoda: Naticidae), in the White and Barents seas (Chubrik, 1966; Timofeeva, 1976). We obtained *Lecithaster* maritae from various fish species, metacercariae from planktonic copepods and intramolluscan lifecycle stages from *C. affinis*, and compared them by 28S ribosomal DNA (rDNA) and internal transcribed spacer 2 (ITS2) sequencing to determine whether they are the stages of a single life cycle, and to define their species identity.

Material and methods

Sampling was performed at the Keret Archipelago, White Sea, in 2019–2021. We searched for the maritae of *Lecithaster* in 16 fish species. Three species of gastropods from the family

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Naticidae (*C. affinis*, $N = 270$; *Euspira pallida* (Broderip & G. B. Sowerby I, 1829), $N = 24$; *Amauropsis islandica* (Gmelin, 1791), $N = 3$) were dissected to find lecithasterid sporocysts and cercariae. Metacercariae were obtained from planktonic copepods.

Sporocysts, metacercariae and maritae were fixed in 96% ethanol for the whole mounts. All specimens of maritae were heat-killed before fixation. Worms were stained with acetocarmine (Sigma-Aldrich, Steinheim, Germany) followed by destaining in 0.1 M hydrogen chloride in 70% ethanol, dehydrated in a graded alcohol series, clarified in xylol and mounted in Canada balsam. Cercarial gross morphology was studied on temporary mounts of live specimens or some fixed in 2.5% glutaraldehyde in sea water. Whole mounts were observed under microscopes Leica DM 500 or DM 2500 (Leica Microsystems, Wetzlar, Germany); photos and videos were taken in bright field and with differential interference contrast using a Nikon DS Fi1 (Nikon, Tokyo, Japan) or with a smartphone camera. Measurements were made using Fiji software (Schindelin et al., 2012). All the measurements are in micrometres.

For scanning electron microscopy (SEM), 2.5% glutaraldehyde-fixed cercariae were rinsed in distilled water, dehydrated in ethanol and acetone, dried in a critical-point dryer, coated with platinum and examined with a Quanta 250 (FEI, Eindhoven, Netherlands) at 15 kV. Visualization of musculature and flame cells arrangement was performed by means of confocal laser scanning microscopy (CLSM). Specimens were fixed in 4% paraformaldehyde in 0.01 M phosphate-buffered saline and stained with TRITC-labelled phalloidin and DAPI (Sigma-Aldrich, Steinheim, Germany). The protocol was the same as described in Kremnev et al. (2020).

Ethanol-fixed sporocysts, metacercariae and maritae were used for molecular studies. To extract DNA we took a fragment of metacercaria, marita (anterior part of the oral sucker or a piece from posterior body end) or sporocyst. The samples were taken from 96% ethanol and dried completely, incubated in 200 µl of 5% solution of Chelex® 100 resin (Bio-Rad, Hercules, California, USA) with 0.2 mg/ml of proteinase K (Evrogen, Moscow, Russia) at 56°C for 4 h, then kept for 8 min at 90°C and centrifuged for 10 min at 14,000 rpm. The supernatant was then transferred into a new tube and stored at −20°C.

Amplification of the 28S rDNA and ITS2 region was performed with primers digl2 (5′-AAGCATATCACTAAGCGG-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) for 28S (domains D1–D3) (Tkach et al., 1999; Olson et al., 2003), and with primers 3S (5′-GTACCGGTGGATCACGTGGCTAGTG-3′) and ITS2.2 (5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′) for ITS2 (Morgan & Blair, 1995). The 20 µl reaction mixture contained 4 µl of ScreenMix-HS (Evrogen), 0.5 µl of forward and reverse primers (10 pmol/µL), 2 µl of the DNA and 13 µl of polymerase chain reaction (PCR)-grade water (Evrogen). PCRs were run on a T100 Thermal Cycler (Bio-Rad, USA) with the following conditions: 40 cycles of 30 s at 95°C, 30 s at 54°C and 60 s at 72°C for 28S, and 35 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C for ITS2. In all reactions initial denaturation was at 95°C for 5 min and final extension was at 72°C for 10 min. PCR products were stained with ethidium bromide and visualized through electrophoresis on a 1% agarose gel. Sequencing was carried out with PCR primers on an ABI Prism 3500xl genetic analyser (Applied Biosystems, Foster City, California, USA). To analyse and edit chromatograms, and to build alignments we used Geneious Prime 2022.0.1 (https://www.geneious.com). The Basic Local Alignment Search Tool (BLAST) was used for preliminary assessment of newly generated sequences. Relevant data for alignments were obtained from GenBank. To annotate 28S and ITS2, we used the sequence of *Aponurus* sp. (HQ713442; Carreras-Aubets et al., 2011) as a reference. Pairwise genetic distances between species (as the number of base differences per site) were calculated in MEGA 7 (Kumar et al., 2016). The maximum likelihood (ML) analysis was conducted at the CIPRES Science Gateway (https://www.phylo.org) using RAxML (Stamatakis, 2014) with 1000 bootstrap iterations. The model of nucleotide substitution was estimated as GTR + G for the 28S dataset and TVM + I for the ITS2 dataset using jModelTest (Darriba et al., 2012) at the CIPRES Science Gateway.

Results

Maritae of the genus *Lecithaster* were found in nine fish species: Atlantic wolffish *Anarhichas lupus* Linnaeus, 1758; Pacific herring *Clupea pallasii* Valenciennes, 1847; European whitefish *Coregonus lavaretus* (Linnaeus, 1758); navaga *Eleginus nawaga* (Walbaum, 1792); Atlantic cod *Gadus morhua* Linnaeus, 1758; common dab *Limanda limanda* (Linnaeus, 1758); Shorthorn sculpin *Myoxocephalus scorpius* (Linnaeus, 1758); pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792); and rainbow smelt *Osmerus mordax dentex* Steindachner & Kner, 1870. Two specimens of *Lecithaster* metacercariae were obtained from the planktonic copepods *Pseudocalanus* sp. and *Metridia longa* (Lubbock, 1854). In the gastropods *C. affinis* we found lecithasterid sporocysts and cercariae consistent with those described as *C. saccocaudata* (Chubrik, 1966; Timofeeva, 1976). Two other naticid gastropods, *E. pallida* and *Amauropisis islandica*, were free from lecithasterid infection.

Molecular analysis

For the molecular analysis we used 19 isolates (listed in table 1). We obtained 1068 bp-long fragments of 28S rDNA for 17 specimens from the definitive and intermediate hosts. The closest 24 BLAST hits were the members of the genus *Lecithaster*, and they were included into an alignment to estimate conspecificity. After trimming ends to fit the shortest sequence, the alignment was 980 bp long. According to the matrix of pairwise distances, our specimens grouped together (see supplementary table S1), though one substitution was present: three maritae (isolates A24.2, D3.2 and D6.1) differed from the rest in the position 368 (C/T). This variation was not consistent with the host species. Our specimens were close to *L. gibbosus* (AY222199, Olson et al., 2003) and *Lecithaster salmonis* Yamaguti, 1934 (MH625979–81, Atopkin et al., 2020), with pairwise distances below 0.0031 ± 0.0018 (1–3 substitutions). The maximal number of substitutions within previously sequenced species of *Lecithaster* was three – in *Lecithaster confusus* (MH628305–6, Sokolov et al., 2019). Pairwise distances between the sequences of different species of the *Lecithaster* (excluding *L. gibbosus* and *L. salmonis*) varied from 0.0115 ± 0.0040 to 0.1250 ± 0.0345 (12 to 113 substitutions).

For the ML analysis we removed all the identical sequences; thus, the alignment included 12 specimens of *Lecithaster*. *Merlucciotrema praeclarum* (AY222204, Olson et al., 2003) was added as an outgroup. The alignment was 980 bp long after trimming to the shortest sequence and removing gaps created by *M. praeclarum*. In the resulting tree, our sequences, together with those of *L. gibbosus* and *L. salmonis*, formed a well-supported branch (fig. 1). Within it interrelationships were unresolved.

For 19 specimens we also obtained a 574–644 bp fragment including partial 5.8S rDNA (104–143 bp), complete ITS2 and

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Table 1. Isolates, their origin and GenBank accession numbers for sequences.

			GenBank accession numbers		
ID	Stage	Host species	28S	5.8S + ITS2	Hologenophore
A24.2	Marita	*Eleginus nawaga*	OM850370	OM850397	VA24.2
A24.6	Marita	*Limanda limanda*	OM850371	OM850398	VA24.6
D3.1	Marita	*Coregonus lavaretus*	OM850372	OM850399	VD3.1
D3.2	Marita	*Osmerus mordax dentex*	OM850373	OM850400	VD3.2
D3.4	Marita	*Anarhichas lupus*	N/A	OM850401	VD3.4
D3.5	Marita	*Gadus morhua*	OM850374	OM850402	VD3.5
D6.1	Marita	*E. nawaga*	OM850375	OM850403	VD6.1
D6.2	Marita	*E. nawaga*	OM850376	OM850404	VD6.2
D6.5	Marita	*Myoxocephalus scorpius*	OM850377	ON320555	VD6.5
D11.5	Marita	*L. limanda*	OM850378	OM850405	VD11.5
D11.6	Marita	*L. limanda*	OM850379	OM850406	VD11.6
D12.8	Marita	*Oncorhynchus gorbuscha*	OM850380	ON320556	VD12.8
G8.7	Marita	*O. gorbuscha*	N/A	OM850407	VG8.7
D10.2	Metacercaria	*Pseudocalanus* sp.	OM850381	OM850408	VD10.2
D10.3	Metacercaria	*Metridia longa*	OM850382	OM850409	N/A
D4.5	Sporocyst	*Cryptonatica affinis*	OM850383	OM850410	N/A
D4.6	Sporocyst	*C. affinis*	OM850384	OM850411	N/A
D4.8	Sporocyst	*C. affinis*	OM850385	OM850412	N/A
D4.9	Sporocyst	*C. affinis*	OM850386	OM850413	N/A

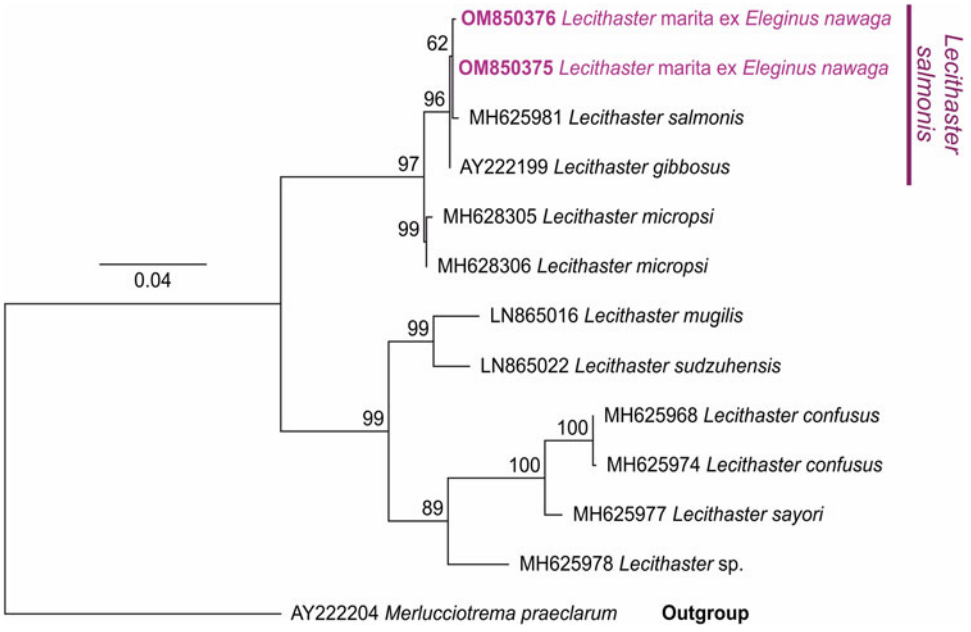

Fig. 1. Phylogenetic affinities of the White Sea *Lecithaster* maritae based on the 28S rDNA sequence data, inferred with the ML method. Newly generated sequences are indicated in bold and purple. Bootstrap values are printed in nodes. Scale bar shows the substitution rate. Identification of a group of sequences as *Lecithaster salmonis* is justified in Results and Discussion.

the beginning of 28S (20–62 bp) (table 1). From GenBank, comparable data were available for only five species of *Lecithaster*: *L. confusus* (MH625982–90), *L. sayori* (MH625991), *L. salmonis* (MH625993–4), *L. stellatus* (AJ224749) and *Lecithaster* sp. (MH625992) (Anderson & Barker, 1998; Atopkin et al., 2020). We excluded *L. stellatus* from the analysis as the sequence was

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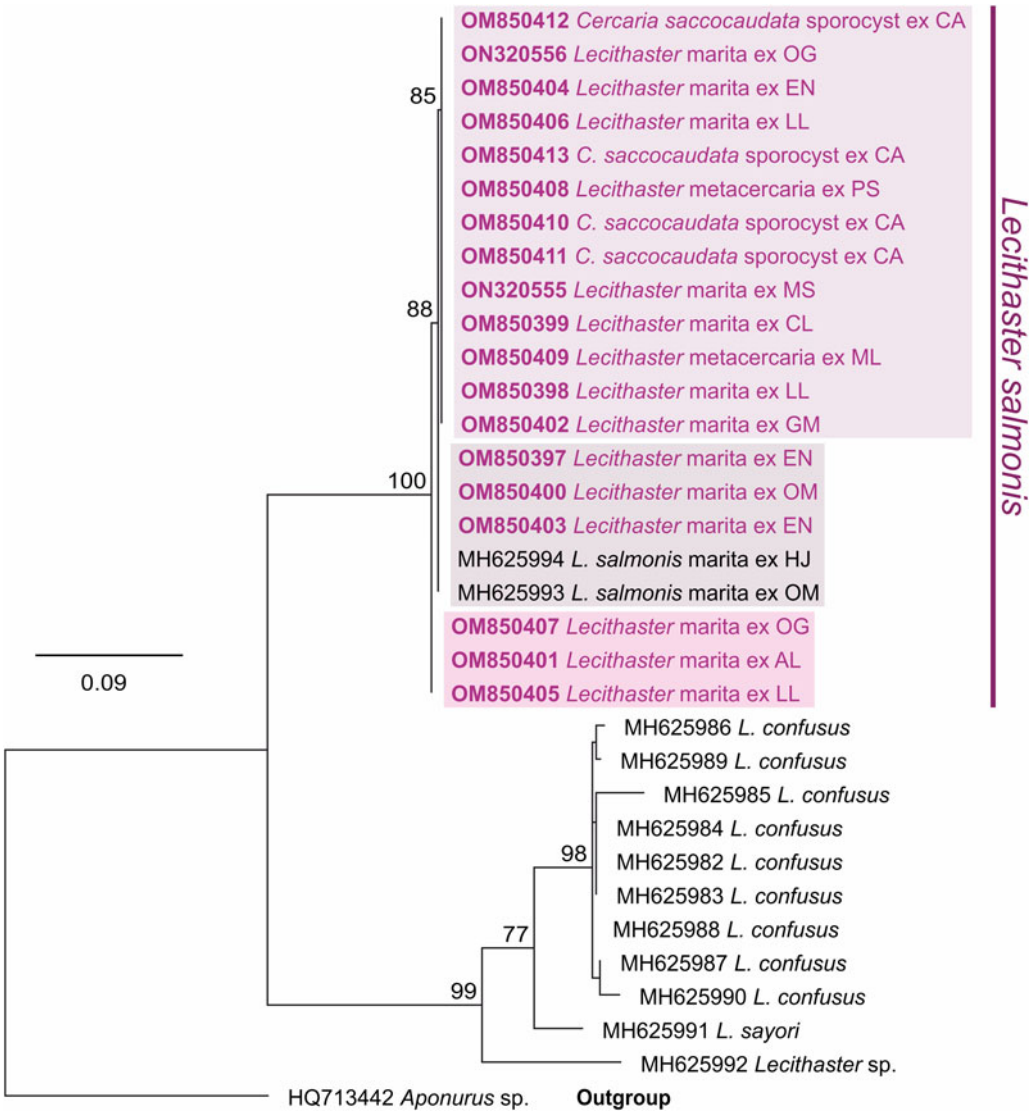

Fig. 2. Phylogenetic affinities of the White Sea *Lecithaster* lifecycle stages based on the ITS2 sequence data, inferred with the ML method. Newly generated sequences are indicated in bold and purple. Bootstrap values are printed in nodes. Scale bar shows the substitution rate. Identification of a group of sequences as *Lecithaster salmonis* is justified in Results and Discussion. Abbreviations: AL, *Anarhichas lupus*; CA, *Cryptonatica affinis*; CL, *Coregonus lavaretus*; EN, *Eleginus nawaga*; GM, *Gadus morhua*; HJ, *Hypomesus japonicus*; LL, *Limanda limanda*; ML, *Metridia longa*; OG, *Oncorhynchus gorbuscha*; OM, *Osmerus mordax dentex*; PS, *Pseudocalanus* sp.

much shorter at the 5' end than the rest, and clearly differed from our sequences within the overlapping region in 173 positions. *Aponurus* sp. (HQ713442; Carreras-Aubets et al., 2011) was added as an outgroup; gaps created by *Aponurus* sp. were removed. Thus, the alignment consisted of 31 sequences and was 508 bp long, including gaps. Our specimens grouped together, though three variable positions were present among them, resulting in three groups with a maximal pairwise distance of 0.0061 ± 0.0046 (fig. 2; supplementary table S2). One of these groups differed from *L. salmonis* in one ambiguous position (distance = 0), and the others in 2–3 positions, including one ambiguity (maximal pairwise distance = 0.0040 ± 0.0037). The heterogeneity among our specimens was not consistent with host species. The highest intraspecific distance was in *L. confusus*: 0.0430 ± 0.0213 (18 positions). The minimal interspecific pairwise distance was 0.0590 ± 0.0275 (39 positions).

Descriptions

Maritae

Locality. White Sea, Keret Archipelago, Vichennaya Luda Island, Malyi Gorelyi Island, Matryonin Island.

Hosts and infection rates. Anarhichas lupus (prevalence 11%, *N* = 27; mean intensity (MI) = 3.7); *C. pallasii* (14%, *N* = 37; MI = 1); *C. lavaretus* (25%, *N* = 4; MI = 2); *E. nawaga* (8%, *N* = 24; MI = 5.5); *G. morhua* (8%, *N* = 125; MI = 8.4); *L. limanda* (4%, *N* = 115; MI = 3.8); *M. scorpius* (5%, *N* = 91; MI = 2.2); *O. gorbuscha* (100%, *N* = 3; MI = 85.7); *O. mordax dentex* (30%, *N* = 20; MI = 4.7).

Sites. Midgut, pyloric ceca, hindgut, stomach.

The measurements and principal morphological characteristics of the hologenophores are given in table 2. As pieces of the maritae were taken for DNA extraction, not all of the specimens contributed a data point to all metrical variables. Considering

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Table 2. Measurements of maritae. Specimens are grouped according to their genetic heterogeneity in ITS2. All the specimens are *Lecithaster salmonis* (see Results and Discussion).

Hologenophores	VA24.6 (ex LL), VD3.1 (ex CL), VD3.5 (ex GM), VD6.2 (ex EN), VD6.5 (ex MS), VD12.8 (ex OG)	VD3.4 (ex AL), VG8.7 (ex OG), VD11.5 (ex LL)	VA24.2 (ex EN), VD6.1 (ex EN)
Body length (BL)	1063 (725–1222)	879 (830–913)	1231[a]
Body maximum width	350 (273–401)	271 (269–273)	360 (298–422)
Forebody (FO)	228 (151–315)	178 (160–193)	326 (267–384)
FO/BL (%)	22 (20–26)	20 (18–23)	31[a]
Post-caecal region (PC)	130 (63–248)	113 (92–134)	125[a]
PC/BL (%)	12 (5–21)	13 (11–15)	10[a]
Preoral lobe length	17 (11–20)	17[a]	18 (12–24)
Oral sucker	94 (86–110) × 109 (95–131)	92 (87–96) × 103 (98–108)	116 (100–132) × 115 (112–117)
Ventral sucker	206 (166–246) × 208 (170–246)	190 (162–228) × 186 (170–211)	196 (177–214) × 194 (170–218)
Sucker length ratio	1.94 (1.93–1.95)	2.23 (2.08–2.38)	1.70 (1.62–1.77)
Pharynx	71 (50–83) × 77 (63–85)	77 (66–98) × 70 (62–86)	63 (62–63) × 64 (57–70)
Genital pore position	Middle of forebody; median/submedian	Middle of forebody; median	Middle of forebody; median/ submedian
Sinus sac	77 (49–111) × 49 (34–58)	62 (56–70) × 39 (39–39)	59 (45–73) × 39 (33–44)
Pars prostatica length (PP)	187 (152–221)	182 (146–205)	212 (201–223)
PP/BL (%)	18 (13–28)	21 (18–22)	16[a]
Pars prostatica position	Anterior to ventral sucker; expands up to 1/3, 1/2, 2/3 or posterior border of ventral sucker	Expands up to 1/2 or 1/3 of ventral sucker	Anterior to ventral sucker, expands up to 1/2 of ventral sucker
Seminal vesicle	148 (109–181) × 75 (57–104)	157 (88–203) × 85 (66–100)	166 (152–179) 35 × (33–36)
Seminal vesicle position	At the level of ventral sucker, expands posterior to ventral sucker; posterior to ventral sucker	At the level of ventral sucker, expands posterior to ventral sucker	At the level of ventral sucker, expands posterior to ventral sucker
Testes shape	Entire	Entire	Entire
Left testis	89 (60–114) × 73 (51–97)	124 (69–151) × 88 (63–106)	99 (93–105) × 90 (77–102)
Right testis	89 (57–113) × 78 (48–128)	111 (79–136) × 86 (53–103)	100 (94–105) × 81 (65–96)
Ovary	174 (135–201) × 160 (140–190)	174 (146–191) × 168 (125–192)	193 × 202[a]
Ovary shape	Four-lobed	Four-lobed	Four-lobed
Seminal receptacle	148 (83–228) × 77 (47–130)	108 (83–125) × 75 (57–93)	104 (73–134) × 71 (57–85)
Seminal receptacle position	At the level of ovary, expands anterior to ovary	At the level of ovary, expands anterior to ovary	Expands anterior to ovary[a]
Vitellarium	198 (137–250) × 148 (103–173)	196 (185–214) × 180 (137–203)	225 × 217[a]
Vitellarium position	Postovarian	Postovarian	Postovarian[a]
Post-vitelline region (PV)	93 (77–112)	73 (69–76)	141[a]
PV/BL (%)	9 (7–11)	8	11[a]
Uterus	Expands posterior to vitellarium	Expands posterior to vitellarium	Expands posterior to vitellarium
Eggs	20 (19–22) × 14 (13–14)	20 (19–20) × 13 (13–14)	20 (19–21) × 13 (12–15)

[a]Data based on one specimen. Abbreviations: AL, *Anarhichas lupus*; CL, *Coregonus lavaretus*; EN, *Eleginus nawaga*; GM, *Gadus morhua*; LL, *Limanda limanda*; MS, *Myoxocephalus scorpius*; OG, *Oncorhynchus gorbuscha*.

heterogeneity in the sequenced ITS2 and 28S rDNA fragments, we give characteristics of three groups of genetically different maritae separately. Immature specimens (without eggs in the uterus) were not considered.

There were no clear morphological differences consistent with the genetic heterogeneity in our specimens. Schemes of the hologenophores (fig. 3) are given to illustrate the position and size of *pars prostatica* and seminal vesicle and the shape of vitellarium lobes – the three most variable characteristics among the studied maritae. Our specimens were most similar to descriptions of *L. salmonis*, *L. confusus* and *L. gibbosus*, on the basis of *pars prostatica* length, the position of the seminal vesicle and the

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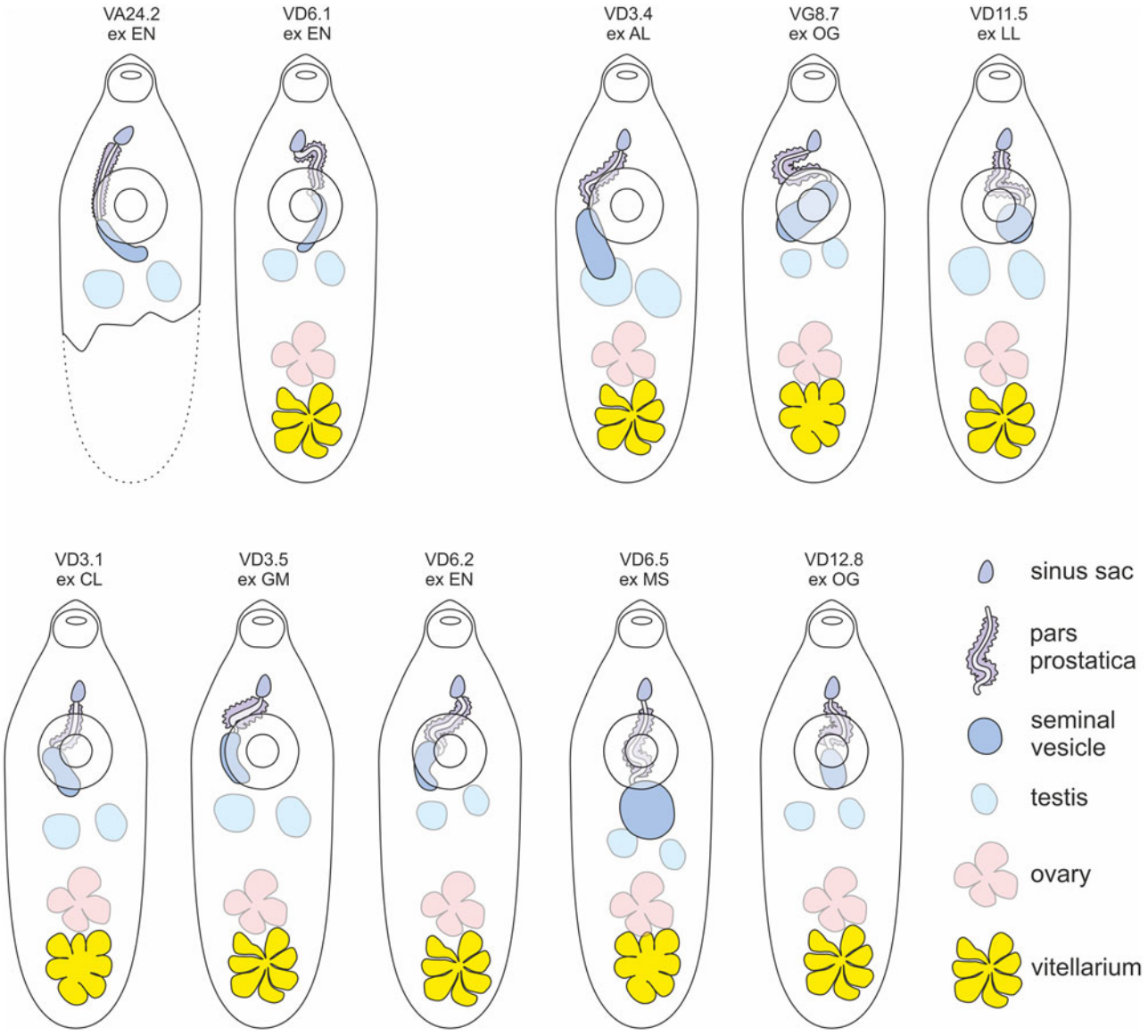

Fig. 3. Main morphological characters of the hologenophores of *Lecithaster* maritae from the White Sea. Specimens are grouped according to their genetic heterogeneity in ITS2. Abbreviations: AL, *Anarhichas lupus*; CL, *Coregonus lavaretus*; EN, *Eleginus nawaga*; GM, *Gadus morhua*; LL, *Limanda limanda*; MS, *Myoxocephalus scorpius*; OG, *Oncorhynchus gorbuscha*.

shape of vitellarium lobes. However, they were apparently different from *L. gibbosus* and *L. confusus* in egg size: 19–22 × 12–15 (our data) vs. 21–36 × 13–18 in *L. gibbosus* (Odhner, 1905; Manter, 1926; Linton, 1940; Zhukov, 1960; Shimazu, 2018) and 12–20 × 7–13 in *L. confusus* (Odhner, 1905; Linton, 1940).

Taking into account both molecular and morphological data, we consider that all the maritae studied here pertain to *L. salmonis*.

Metacercaria

Based on one hologenophore

Host. *Pseudocalanus* sp.

Locality. White Sea, Keret Archipelago, Vichennaya Luda Island.

Body 568 × 226; forebody 150 (26% of body length); postvitelline region 103 (18% of body length); post-caecal region 40 (7% of body length); oral sucker 63 × 75, ventral sucker 136 × 147, sucker length ratio 1:2.16; genital pore at middle of forebody, median; sinus sac 41 × 34; *pars prostatica* 118 (21% of body length), reaching middle of ventral sucker; seminal vesicle 152 × 63, extending posterior to ventral sucker; testes entire, left 68 × 70, right 46 × 63; ovary four-lobed 106 × 181; vitellarium postovarian, with seven oval lobes, 129 × 166.

Intramolluscan stages

Host. *Cryptonatica affinis*.

Locality. White Sea, Keret Archipelago, Vichennaya Luda Island, Matryonin Island.

Prevalence. 14.8% ($N = 270$).

Sites. Reproductive and digestive glands.

Sporocysts measurements based on six ethanol-fixed specimens. Sporocysts very long, 4312 (3101–5583) × 111 (90–122). Body with several constrictions. Birth pore terminal at anterior body end.

Cercariae measurements based on 24 infective and 22 pre-infective glutaraldehyde-fixed specimens. Cercariae of cystophorous

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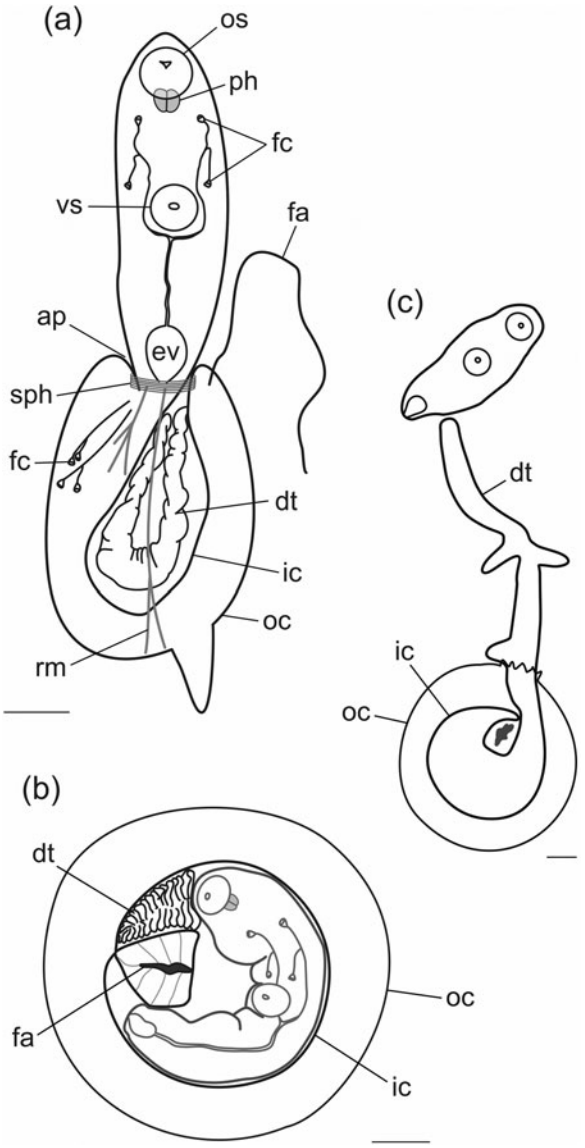

Fig. 4. Schemes illustrating structure of the cercariae, pre-infective (a), infective (b) and with delivery tube everted (c). Scale bars: 20 μm. Abbreviations: ap, caudal cyst aperture; dt, delivery tube; ev, excretory vesicle; fa, filamentous appendage; fc, flame cell; ic, inner cyst layer; oc, outer cyst layer; os, oral sucker; ph, pharynx; rm, retractor muscles; sph, sphincter; vs, ventral sucker.

type, with tail transformed into two-layered caudal cyst (figs 4 and 5). Cyst with delivery tube providing infection of second intermediate host and filamentose appendage 80 (64–97) long. In pre-infective cercaria caudal cyst oval, 78 (68–88) × 54 (43–67); outer cyst layer folded, often forming triangular projection; delivery tube withdrawn inside cavity, filamentose appendage and body outside. Body 108 (89–133) × 33 (29–37), oral sucker 16 (14–19) × 18 (16–20), pharynx 7 (6–8) × 9 (7–10), ventral sucker 13 (11–15) × 16 (12–18). Excretory vesicle Y-shaped, wider distal part 15 (13–17) × 9 (7–10). Two pairs of flame cells present within the body. Two excretory ducts inside caudal cyst, each with pair of flame cells (figs 4a and 5c). When getting into seawater cercariae become infective: body pulls into cyst through aperture by retractor muscles; aperture closes by sphincter muscle (figs 4a and 5c, e); filamentous appendage slowly withdraws into cyst, outer cyst layer inflates (figs 4b and 5d–f). Infective cercariae immobile, outer cyst 113 (109–120) in diameter, 60 (54–64) thick, inner cyst crescent-shaped, 77 (73–82) in diameter, 41 (38–43) thick. Flame cells within caudal cyst absent. Delivery tube measured in live specimens when everted under cover glass pressure ($N = 7$) 218 (201–225) long, with three lateral projections near base (figs 4c and 5g). Width between first and second projections 20 (17–22).

Discussion

Previously, two species of the genus *Lecithaster* have been recorded from the White Sea: *L. gibbosus* and *L. confusus* (Shulman & Shulman-Albova, 1953; Mitenev & Karasev, 2005). Molecular data showed that all the maritae of *Lecithaster* which we found in the White Sea fish likely belong to a single species, despite some variation in 28S rDNA and ITS2. Genetically, this species matched isolates of *L. salmonis* from the Far East (Atopkin et al., 2020) and one isolate of *L. gibbosus* from the northern Atlantic (Olson et al., 2003). At the same time, the difference between our specimens and *L. confusus* from the Far East (Atopkin et al., 2020) was extremely high.

The morphological characteristics are variable among our isolates. They demonstrate certain similarities with *L. salmonis*, *L. gibbosus* and *L. confusus* in vitellaria shape, length and position of the *pars prostatica* and seminal vesicle. Additionally, the morphological variability of our specimens is consistent neither with their genetic heterogeneity, nor with host species. Among the metrical characteristics, we consider the egg size to be the most reliable as it does not depend on fixation procedure, which had not been standardized until recently. The eggs in our specimens are longer than ones of *L. confusus*: 19–22 (our data) vs. 15–17 (Odhner, 1905), 12–20 (Linton, 1940). They are slightly smaller or similar to the eggs of *L. salmonis*: 22–24 (Yamaguti, 1934, 1940), 19–24 (Atopkin et al., 2020). Finally, *L. gibbosus* has a clearly bigger egg size: 25–27 (Odhner, 1905), 23–26 (Manter, 1926), 24–36 (Linton, 1940), 21–27 (Shimazu, 2018).

Considering both molecular and morphological data, we conclude that the White Sea *Lecithaster* is probably *L. salmonis*, which appears to be remarkably variable in several important characteristics commonly used to define species of *Lecithaster*. We suppose that the sequence AY222204 (Olson et al., 2003) from the Atlantic was attributed to *L. gibbosus* erroneously due to this morphological variability, and the egg size might not have been considered.

Lifecycle data are also informative for species recognition. We have discovered intermediate hosts of *L. salmonis* by matching rDNA sequences of maritae to those of the larval lecithasterids. These were sporocysts and cercariae (previously called *C. saccocaudata*) from the gastropod *C. affinis* (Caenogastropoda) and metacercariae from the copepods *Pseudocalanus* sp. and *M. longa*. *Cryptonatica affinis* is present in Japanese waters (Chen & Nomaki, 2021), the type locality of *L. salmonis*, further supporting the identification of this species. On the contrary, the first intermediate hosts of *L. confusus* and *L. gibbosus* are heterobranch gastropods from the family Pyramidellidae (Hunninen & Cable, 1943; Køie, 1989), which are absent in the White Sea. Thus, we consider the records of *L. confusus* and *L. gibbosus* from the White Sea to be erroneous. *Lecithaster* maritae recovered from this region in previous studies (Shulman & Shulman-Albova, 1953; Mitenev & Karasev, 2005) probably belong to the species *L. salmonis*.

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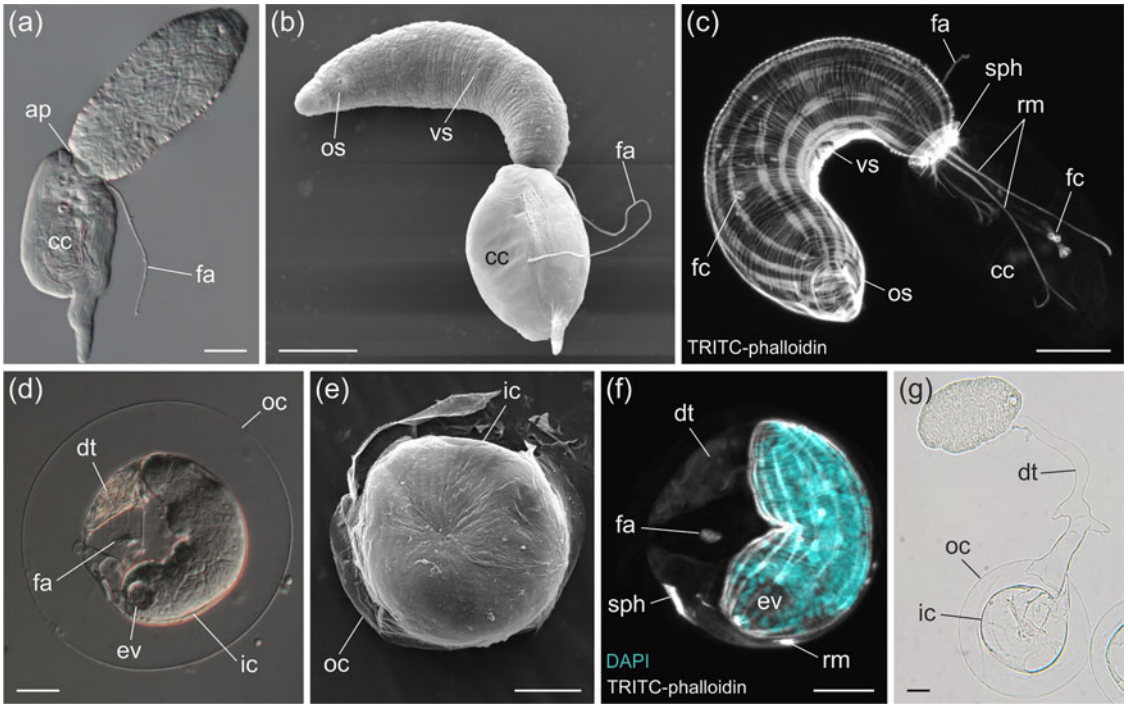

Fig. 5. Structure of the cercariae. (a–c) Pre-infective cercariae, differential interference contrast (a), SEM (b) and TRITC-phalloidin staining, CLSM (c). (d–f) Infective cercaria, differential interference contrast (d), SEM with outer cyst layer partially torn (e), TRITC-phalloidin and DAPI staining, CLSM (f). (g) Cercariae with delivery tube everted, bright field. Scale bars: 20 μm. Abbreviations: ap, caudal cyst aperture; cc, caudal cyst; dt, delivery tube; ev, excretory vesicle; fa, filamentous appendage; fc, flame cell; ic, inner cyst layer; oc, outer cyst layer; os, oral sucker; ph, pharynx; rm, retractor muscles; sph, sphincter; vs, ventral sucker.

We found *L. salmonis* maritae in nine fish species, and Shulman & Shulman-Albova (1953) recorded it from seven more. The host range covers distant taxonomic groups of teleosts and even one lamprey species (Arctic lamprey *Lethenteron camtschaticum* (Tilesius, 1811)). However, among them, Atlantic salmon *Salmo salar* Linnaeus, 1758 seems to be the most successfully colonized, with documented intensity of up to several thousands (Shulman & Shulman-Albova, 1953; Mitenev & Karasev, 2005). Pink salmon *O. gorbuscha* is also highly infected, though the intensity is lower than in *S. salar* (Barskaya et al., 2005; our data). The type host of *L. salmonis* is also a representative of the subfamily Salmoninae Jarocki or Schinz, 1822, chum salmon *Oncorhynchus keta* (Walbaum, 1792) (Yamaguti, 1934). Infection data are not available for this host species.

As planktonic copepods act as the second intermediate hosts of *L. salmonis*, feeding on microplankton is expected to enhance the prevalence and intensity. However, the facts say otherwise: infection rates in fishes besides the Salmoninae do not differ substantially between plankton feeders (e.g. Pacific herring, rainbow smelt), benthic feeders (e.g. Atlantic wolffish, Shorthorn sculpin) and piscivorous fish (e.g. Atlantic cod) (Shulman & Shulman-Albova, 1953; our data). Thus, higher rates of *L. salmonis* infection in the Salmoninae probably result from better host compatibility, approving its specific name. Specificity towards the first intermediate host, *C. affinis*, must be high: similar cercariae have never been found in any other gastropods in the White and Barents seas (Chubrik, 1966; our observations). On the contrary, the range of copepods that may act as second intermediate hosts must be wide, judging from our data and lifecycle studies on the hemiuroid digeneans (Køie, 1979, 1989).

The geographical range of *L. salmonis* known to date includes the boreal Pacific region, the European subarctic (White Sea) and the boreal north-east Atlantic, so it is interrupted. We lack data on a variable DNA marker (e.g. *cox1* gene) that could reveal the expansion route of this species, but some speculations are possible considering the distribution of its hosts. First, the dispersal abilities of parasites largely depend on their most vagile host (Esch et al., 1988; Blasco-Costa & Poulin, 2013), and that is fish in the case of *L. salmonis*. Second, the expansion must have proceeded through the marine environment, and specifically through the Arctic Ocean considering the distribution of the first intermediate host, *C. affinis* (WoRMS, 2022b). The simplest scenario of trans-Arctic transfer would involve some anadromous Arctic Salmoninae with a geographic range overlapping that of salmon in the Pacific and Atlantic. A good candidate is Arctic charr *Salvelinus alpinus* (Linnaeus, 1758), which has circumpolar distribution (Klemetsen et al., 2003). With this host the expansion of *L. salmonis* could go both east to west and vice versa. Direct transfer of this parasite from *Oncorhynchus* (Pacific) to *Salmo* (Atlantic) is unlikely, because the former was introduced into the European sector of the Arctic quite recently, in the 1950s (Crawford & Muir, 2008). Noteworthy, only the eggs were translocated; thus, *Oncorhynchus* spp. could not bring parasites of their own, and were colonized by the local ones (Alekseev et al., 2019). More opportunities for trans-Arctic dispersal could be provided by the other definitive hosts of *L. salmonis*, which migrated from the Pacific towards the Atlantic during the postglacial period: Pacific herring, rainbow smelt and Arctic lamprey (Makhrov & Lajus, 2018).

To summarize our data, the digenean trematode *L. salmonis* was found in the White Sea, though it had previously been known only from the Pacific. Maritae of *L. salmonis* are highly variable in morphology and can be easily misidentified as other species of *Lecithaster*. In the White Sea, they have previously

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been recorded as *L. confusus* and *L. gibbosus*. The morphological variability of *L. salmonis* makes us doubt the principles of species delineation in the genus *Lecithaster*. Thus, it is necessary to study other *Lecithaster* species from their type localities and type hosts using molecular genetic methods. Also, intriguing questions to explore in the future refer to the expansion of *L. salmonis* through the Pacific, Arctic and Atlantic. To test if the geographic range of *L. salmonis* is truly interrupted, intense sampling throughout the Arctic is needed, accompanied by the molecular analysis of *cox1* or another variable gene sufficient for the elucidation of intraspecific genetic structure.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X22000281

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Conflicts of interest. None.

Ethical standards. This study was conducted in compliance with all institutional, national and international guidelines on the care and use of animals.

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