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Infection status of commercial fish with cystacanth larvae of the genus *Corynosoma* (Acanthocephala: Polymorphidae) in Hokkaido, Japan

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ABSTRACT

Acanthocephalans of the genus *Corynosoma* are known as intestinal parasites, mainly of pinnipeds. Human corynosomiasis has been reported as an infrequent foodborne disease in Hokkaido, the northernmost island of Japan. Potential sources of the human infection are marine fish, because they are paratenic hosts of these parasites. In this study, the prevalence and intensity of larval *Corynosoma* in commercial fish from 17 fishing ports of Hokkaido were examined from April 2016 to January 2019. Out of a total of 1217 fish examined, 122 (10.0%) were infected with cystacanth larvae. The infected fish assemblage was composed of 7 families and 13 species from all the coastal seas of Hokkaido (the Pacific Ocean, Okhotsk Sea, and Japan Sea), showing that commercial fish can be source of human infection when eaten raw. Flatfish of the family Pleuronectidae showed the highest intensity of cystacanths, ranging from 1 to 56. A DNA barcoding system was developed in this study, based on the standard mitochondrial *cox1* sequences of morphologically identified adults of *Corynosoma* spp. from pinnipeds in Hokkaido. By using the DNA barcoding, most of the fish-derived cystacanths were identified as either *C. strumosum* or *C. villosum*, and furthermore, a clinical isolate from human as *C. villosum*. Both of the species were commonly detected from various fish of Hokkaido, irrespective of the coastal seas. Flatfish frequently harbored *C. villosum*. Considering the wide range of commercial fish in Hokkaido and the advanced transportation system of fresh fish, there is a possibility that human corynosomiasis will occur everywhere in Japan.

1. Introduction

Acanthocephalans of the genus *Corynosoma* Lühe 1904 (Polymorphidae) are known as intestinal parasites of marine mammals and seabirds, particularly of pinnipeds (Delyamure, 1968; Van Cleave, 1953b; Yamaguti, 1963). The parasite life cycle is based on predator-prey interactions in the marine habitat. Amphipod crustaceans serve as intermediate host. After ingesting the parasite eggs, the development of the larval stages (acanthor, acanthella, and cystacanth) occurs in the hemocoel. Fish acquire the cystacanths through ingesting amphipods. The cystacanths, especially in fish, become the source of infection to the definitive hosts. Thus, fish play a very important role in the transmission of *Corynosoma* as paratenic host (Crompton and Nickol, 1985).

It has been generally assumed that human is an unsuitable host for *Corynosoma*, because only one case of infection with immature *Corynosoma strumosum* has been reported from Alaska (Schmidt, 1971). However, recent reports of human corynosomiasis from Japan showed that intestinal adults can reach sexual maturity (i.e. laying eggs), suggesting humans to be a suitable definitive host (Fujita et al., 2016; Takahashi et al., 2016). To date, three cases of corynosomiasis have been confirmed in Hokkaido, the northernmost island of Japan. All the patients complained of persistent abdominal pain, and the parasites were removed by endoscopy (Fujita et al., 2016; Takahashi et al., 2016; Yagi et al., 2002). The parasites were morphologically identified as *Corynosoma villosum* in the first case (Yagi et al., 2002) and as *Corynosoma* sp. and *C. villosum* in the second case (Fujita et al., 2016). The morphological observation of the whole worm was impossible in the third case. However, a mitochondrial DNA sequence (*cox1*, cytochrome

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c oxidase subunit 1) amplified from the paraffin-embedded parasite tissue of the patient was identical to that of *Corynosoma validum* (Takahashi et al., 2016). Traditional Japanese foods consisting of sliced raw fish, namely "sashimi" and "sushi", are most likely responsible for the accidental infection of the patients.

Hokkaido is surrounded by three bodies of water, namely the Pacific Ocean, Okhotsk Sea, and Japan Sea. The cystacanth larvae of *Corynosoma* have long been noticed in marine fish from Hokkaido. Fujita (1921) detected *Corynosoma osmeri* from the sishamo smelt (*Spirinchus lanceolatus*) in Hidaka (Pacific Ocean), but this species is now synonymized to *C. strumosum* (Van Cleave, 1953a, 1953b). Araki and Machida (1987) further found cystacanths from several species of fish in Samani (Pacific Ocean). These larvae were morphologically identified as *C. strumosum* from the Japanese surf smelt (*Hypomesus japonicus*), the great sculpin (*Myoxocephalus polyacanthocephalus*), the black edged sculpin (*Gymnocanthus herzensteini*), and the barfin flounder (*Verasper moseri*), and as *C. villosum* from the threestripe rockfish (*Sebastes trivittatus*) and the Pacific halibut (*Hippiglossus stenolepis*). These old reports showed both *C. strumosum* and *C. villosum* to be distributed off the Pacific coast of Hokkaido.

Pinnipeds are the principal definitive host of *Corynosoma*, and to a lesser extent, cetaceans are involved. Pinnipeds of Japan are composed of 8 species (Ohdachi et al., 2015), namely the Steller sea lion (*Eumetopias jubatus*), the northern fur seal (*Callorhinus ursinus*), the Japanese sea lion (*Zalophus japonicas*), the Kuril harbor seal (*Phoca vitulina*), the spotted seal (*Phoca largha*), the ringed seal (*Pusa hispida*), the ribbon seal (*Histriophoca fasciata*), and the bearded seal (*Erignathus barbatus*). Most of them seasonally migrate to the coastal seas of Hokkaido from Sakhalin and Kuril Islands (Hoshino et al., 2006; Kenyon and Wilke, 1953; Kobayashi et al., 2009; Kobayashi et al., 2014; Ohdachi et al., 2015). Adults of *C. strumosum* were repeatedly found from Steller sea lions, Kuril harbor seals, spotted seals, and ribbon seals in Hokkaido (Ito et al., 1998; Kaimoto et al., 2018; Nagasawa, 1999; Nakamura et al., 1965; Wakazono, 1953), while *C. villosum* was detected only from northern fur seals captured in the Pacific coastal sea of northern Honshu, the main island of Japan (Machida, 1969). Furthermore, an unidentified species of *Corynosoma* was found from the finless porpoise (*Neophocaena phocaenoides*) in the Pacific coastal sea of central Honshu (Kuramochi et al., 2000; Shiozaki and Amano, 2017). Thus, the mammal surveys also confirmed the distribution of both *C. strumosum* and *C. villosum* in northern seas of Japan, and suggested the difficulty of the morphological identification.

The distribution area and the population size of pinnipeds are affected by direct anthropogenic disturbance and indirect climate change (Kobayashi et al., 2009; Kovacs et al., 2011; Wilke, 1954). Recent increases of Steller sea lions and spotted seals in Hokkaido (Kobayashi et al., 2009, 2014; Mizuno et al., 2018) may influence the infection status of fish with larval *Corynosoma*. Old records of cystacanth-infected fish (Araki and Machida, 1987) are quite insufficient in evaluating the risk of the human infections. Data on the prevalence and intensity of cystacanths in commercial fish are absolutely necessary for the occurrence prediction and reasonable prevention of human corynosomiasis. In this study, a large-scale survey was carried out to examine the infection status of fish from all the coastal seas of Hokkaido. The main purposes of this study are (i) to locate the infection sites of cystacanth larvae, (ii) to estimate the prevalence and intensity of infection, and (iii) to morphologically and molecularly characterize the causative species. A DNA barcoding system was also developed as a tool to correctly identify the species of cystacanths, through a comparison with the *cox1* standard sequences of morphologically identified adults of pinniped-derived *Corynosoma* and seabird-derived *Andracantha* in Hokkaido. The resultant DNA barcode sequences were utilized to infer the population genetic structures of cystacanths.

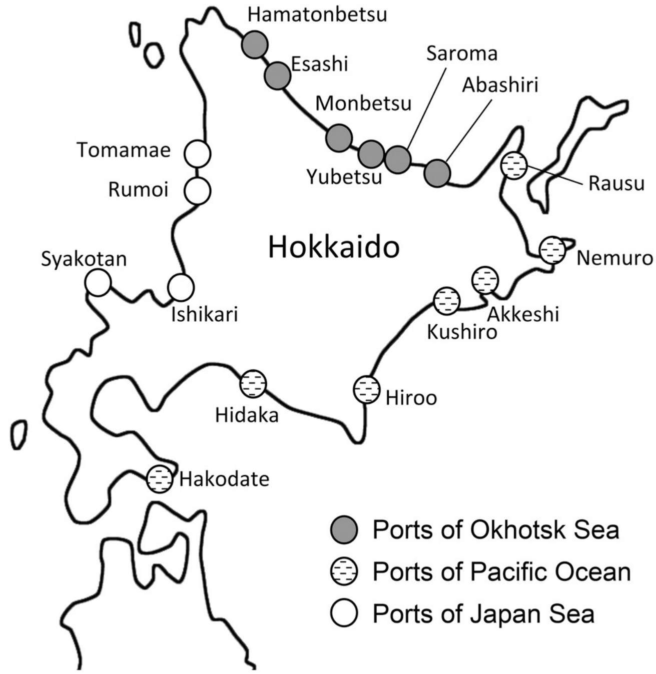

Fig. 1. Fishing ports on the coasts of Hokkaido, the northernmost island of Japan, from which fish samples were collected.

2. Materials and methods

2.1. Sample collections

From April 2016 to January 2019, commercial fish were collected from 17 fishing ports of Hokkaido located on the coasts of the Pacific, Okhotsk Sea, and Japan Sea (Fig. 1). The scientific names of fish used in this paper mainly follow those recommended by Nakabo (2013). The fish were purchased from dealers together with information of their origins, or were received as gifts from fishermen. Samples were individually dissected under a stereomicroscope to find cystacanth larvae, particularly from the visceral surface. The number of cystacanths per each infected fish was counted to measure the intensity of infection. Some of the encysted cystacanths were treated with 0.25% trypsin-EDTA solution (Thermo Fisher Scientific, USA) at 37 °C for 30 min to accelerate the evagination of the upper trunk and proboscis. After removing undigested debris by a small brush-pencil, the excysted larvae were examined under a light microscope. All the encysted and excysted larvae were kept in 70% ethanol until later morphological and molecular identification.

In other research projects, adult acanthocephalans had already been collected during 1999 to 2018 from accidental carcasses of marine mammals and seabirds, mainly in Hokkaido (Supplementary Fig. 1). The ethanol-preserved adult specimens from pinnipeds were used in this study as standards for morphological and molecular identification. The origins of the standard specimens are as follows: *C. strumosum* from one spotted seal in Rebun island (Japan Sea), *C. strumosum* from two spotted seals in Abashiri (Okhotsk Sea), *C. strumosum* from one Kuril harbor seal in Erimo (Pacific Ocean), *C. villosum* from two Steller sea lions in Abashiri (Okhotsk Sea), and *Corynosoma semerme* from one spotted seal in Abashiri (Okhotsk Sea). As well as pinnipeds, seabirds serve as definitive hosts for acanthocephalans of the genera *Andracantha* and *Corynosoma*. The ethanol-preserved adult specimens of *Andracantha phalacrocoracis* from one pelagic cormorant (*Phalacrocorax pelagicus*) in Shibetsu (Pacific Ocean) and one Japanese cormorant (*Phalacrocorax capillatus*) in Erimo (Pacific Ocean) also were used as a standard.

Corynosoma sp. from cetaceans is morphologically very similar to *C.*

Table 1
The prevalence of cystacanth larvae in commercial fish from the coastal seas of Hokkaido, Japan.

Fishes		Collection sites		No. fishes examined	No. fishes infected (%)	No. cystacanths per infected fish Mean (range)
Families	Species	Fishing ports	Coasts			
Osmeridae	*Osmerus dentex*	Abashiri	Okhotsk	12	1 (8.3)	1 (1)
		Akkeshi	Pacific	86	32 (37.2)	2.6 (1–7)
		Nemuro	Pacific	162	40 (24.7)	1.7 (1–4)
	Hypomesus japonicus	Akkeshi	Pacific	240	4 (1.7)	1 (1)
		Nemuro	Pacific	151	5 (3.3)	1.2 (1–2)
	Hypomesus nipponensis	Abashiri	Okhotsk	167	0 (0)	
Clupeidae	*Clupea pallasii*	Esashi	Okhotsk	31	2 (6.4)	1 (1–2)
		Yubetsu	Okhotsk	38	8 (21.1)	1.1 (1–2)
		Saroma	Okhotsk	5	2 (40.0)	1 (1)
		Abashiri	Okhotsk	3	1 (33.3)	3
		Nemuro	Pacific	28	1 (3.5)	1
		Ishikari	Japan Sea	4	0 (0)	
Hexagrammidae	*Pleurogrammus azonus*	Yubetsu	Okhotsk	2	0 (0)	
		Abashiri	Okhotsk	1	1 (100)	5
		Shakotan	Japan Sea	43	1 (2.3)	2
Gadidae	*Gadus macrocephalus*	Abashiri	Okhotsk	3	1 (33.3)	2
		Hiroo	Pacific	10	0 (0)	
	Eleginus gracilis	Abashiri	Okhotsk	13	0 (0)	
Pleuronectidae	*Platichthys stellatus*	Hamatonbetsu	Okhotsk	10	4 (40.0)	5.5 (1 – 12)
	Pleuronectes herzensteini	Monbetsu	Okhotsk	3	0 (0)	
		Rausu	Pacific	10	6 (10.0)	12.8 (3–56)
		Kushiro	Pacific	20	0 (0)	
	Pleuronectes schrenki	Hamatonbetsu	Okhotsk	1	1 (100)	2
		Abashiri	Okhotsk	2	0 (0)	
	Pleuronectes obscurus	Abashiri	Okhotsk	4	0 (0)	
		Rausu	Pacific	7	2 (28.6)	7 (2 – 12)
	Pleuronectes mochigarei	Rumoi	Japan Sea	13	7 (53.8)	4.3 (1–14)
	Microstomus achne	Hidaka	Pacific	2	0 (0)	
	Paralichthys olivaceus	Tomamae	Japan Sea	1	1 (100)	9
Scorpaenidae	*Sebastolobus macrochir*	Rausu	Pacific	1	0 (0)	
		Kushiro	Pacific	3	0 (0)	
	Sebastes trivittatus	Abashiri	Okhotsk	1	0 (0)	
		Nemuro	Pacific	1	1 (100)	4
	Sebastes schlegelii	Abashiri	Okhotsk	3	0 (0)	
	Sebastes glaucus	Rausu	Pacific	4	0 (0)	
	Sebastes taczanowskii	Abashiri	Okhotsk	6	0 (0)	
Cottidae	*Myoxocephalus stelleri*	Hamatonbetsu	Okhotsk	2	0 (0)	
		Abashiri	Okhotsk	9	1 (11.1)	3
	Myoxocephalus brandti	Abashiri	Okhotsk	9	0 (0)	
	Enophrys diceraus	Abashiri	Okhotsk	2	0 (0)	
	Gymnocanthus herzensteini	Abashiri	Okhotsk	2	0 (0)	
	Gymnocanthus intermedius	Abashiri	Okhotsk	1	0 (0)	
Cyclopteridae	*Aptocyclus ventricosus*	Hakodate	Pacific	1	0 (0)	
Cyprinidae	*Tribolodon hakonensis*	Abashiri	Okhotsk	34	0 (0)	
Gasterosteidae	*Gasterosteus nipponicus*	Abashiri	Okhotsk	57	0 (0)	
	Pungitius sp.	Abashiri	Okhotsk	9	0 (0)	
Total			**Pacific**	**726**	**91 (12.5)**	**2.9 (1–56)**
			Okhotsk	**430**	**22 (5.1)**	**2.5 (1–12)**
			Japan Sea	**61**	**9 (14.8)**	**4.5 (1–14)**
Ground total				**1217**	**122 (10.0)**	**2.8 (1–56)**

strumosum, but its body size is considerably larger (Kuramochi et al., 2000). To clarify the specific status, ethanol-preserved adults from one finless porpoise (*Neophocaena phocaenoides*) and two harbor porpoises (*Phocoena phocoena*) were subjected to molecular identification. The former porpoise was from Hokota (Pacific Ocean), central Honshu (Kuramochi et al., 2000), and the latter from Rausu (Pacific Ocean), Hokkaido.

2.2. Morphological observations

An optical microscope with digital imaging apparatus (Axio Imager, Zeiss, Germany) was used for morphological observations. Measurements were taken using the accessory software (AxioVision, Zeiss). Cystacanth larvae from fish were observed alive, or the ethanol-fixed larvae were mounted with Hoyer's medium to observe in detail. Some of the standard specimens of adult acanthocephalans were stained with acetocarmine or Heidenhain's iron hematoxylin, dehydrated in graded ethanol series, cleared in creosote, and mounted with Canada balsam. The standard specimens of adult *Corynosoma* were identified to species, particularly based on the characteristics of the proboscis hooks (numbers of longitudinal rows and of hooks per row) and the distributional pattern of the trunk spines (Golvan, 1959; Lühe, 1911; Nickol et al., 2002; Presswell et al., 2018; Schmidt, 1975; Van Cleave, 1953a, 1953b; Yamaguti, 1963). The vouchers of *A. phalacrocoracis* from pelagic cormorants in Hokkaido (Machida, 1966), which were kept in the National Museum of Nature and Science, Tsukuba, Japan (collection no. NSMT-As1876), were employed to confirm the species identity of the present standard specimens.

2.3. Phylogenetic analyses

Genomic DNA was purified from each of adult acanthocephalans and cystacanth larvae by using a spin column kit (DNeasy Blood & Tissue Kit, Qiagen, Netherlands), and then served as a template for polymerase chain reaction (PCR). The hot-start version of *Ex Taq* DNA polymerase (TaKaRa, Japan) was employed for PCR, together with the manufacturer-supplied reaction buffer. For a distance-based DNA barcoding, the 1 kb fragment of mitochondrial *cox1* was amplified using the original primer set, Cory-CO1/F (5'-TGC TTC GTT GGT TTA TGT CTT TGA-3′) and Cory-CO1/R (5'-CAT ACT TAA CAC ATA ATG AAA ATG-3′). In the case of *Andracantha* spp., the reverse primer was replaced by Cory-CO1/R2 (5'-ACC TGA CAA TTG GAA ATT GCC TGT-3′). An almost complete fragment (approximately 2.7 kb) of nuclear 28S ribosomal DNA (rDNA) was also amplified using the primer set, Acan-LSU/F (5'-CAA GTA CCG TGA GGG AAA GTT GC-3′) and Acan-LSU/R (5'-CTT CGC AAT GAT AGG AAG AGC C-3′). The PCR amplicons were sequenced using BigDye terminator cycle sequencing kit and ABI genetic analyzer 3500 (Applied Biosystems, USA). Each of the PCR primers was used as a sequencing primer. Primer walking was done to determine the 28S rRNA gene sequence.

The nucleotide data sets of *cox1* and 28S rDNA were prepared by the multiple aligner MAFFT (Katoh and Standley, 2013). The sequences of morphologically identified adults of *Corynosoma* spp. and *A. phalacrocoracis* were included in the data sets as standards. The comparative sequences of related taxa were retrieved from DDBJ/ENA/GenBank databases. The data sets of *cox1* and 28S rDNA consisted of 575 and 1267 nucleotide sites, respectively. The best-fit substitutional model of each data set were selected by the genetic software MEGA7 (Kumar et al., 2016), and phylogenetic trees were made by maximum likelihood (ML) method of MEGA7, using HKY + G + I model for *cox1* and K2 + G for 28S rDNA. The robustness of the trees was tested by bootstrapping with 500 replicates.

The pairwise divergence values of *cox1* sequences were computed by MEGA7 for a distance-based DNA barcoding. A network figure of *cox1* haplotypes was illustrated by TCS1.21 (Clement et al., 2000), and population genetics indices were calculated by DnaSP6 (Rozas et al., 2017). The data set of *cox1* used for these analyses was composed of 1023 nucleotide sites.

The DNA sequences of acanthocephalans determined in this study have been deposited into DDBJ/ENA/GenBank databases under the accession numbers LC465307–LC465403 (*cox1*) and LC461963–LC461973 (28S rDNA).

3. Results

3.1. Detection of cystacanths from fish

A total of 1217 commercial fish, encompassing 10 families and 28 species, were examined. Out of them, 122 (10.0%) were infected with cystacanth larvae (Table 1). The infected fish assemblage was composed of 7 families and 13 species. A host specificity of the cystacanths was not observed. The infected fish were from 3, 5, and 3 fishing ports on the coasts of the Pacific Ocean, Okhotsk Sea, and Japan Sea, respectively. The prevalences were 12.5% (Pacific Ocean), 5.1% (Okhotsk Sea), and 14.8% (Japan Sea), showing that the cystacanth infection frequently occurs in all the coastal seas of Hokkaido.

The cystacanths were mostly found alive, encysted on the serosal surface of intestine, liver, and body cavity (Fig. 2). In the case of not fresh fish, some of the cystacanths were found excysted and scattered in the body cavity. The mean intensities of cystacanth infections (number of cystacanths per infected fish) were low in each sea area (Table 1), namely 2.9 (Pacific Ocean), 2.5 (Okhotsk Sea), and 4.5 (Japan Sea). Flatfish of the family Pleuronectidae showed the highest intensity of the cystacanths, ranging from 1 to 56.

The encysted cystacanths could not be morphologically identified to species. In addition, considering a possibility of the existence of morphologically indistinguishable cryptic species, a total of 72 cystacanths randomly selected from each of the fish species or from each of the sea areas were subjected to molecular analyses.

3.2. Molecular phylogenetic clustering and DNA barcoding

The *cox1* sequences of 72 fish-derived cystacanths, 19 morphologically identified adults (8 isolates of *C. strumosum*, 4 of *C. villosum*, 3 of *C. semerme*, and 4 of *A. phalacrocoracis*), and 6 adults of *Corynosoma* sp. from porpoises were used for constructing a phylogenetic tree, together with already published *cox1* sequences (García-Varela and Nadler, 2006; García-Varela and Pérez-Ponce de León, 2008; García-Varela et al., 2013; García-Varela et al., 2017; Hernández-Orts et al., 2017; Lisitsyna et al., 2019; Presswell et al., 2018; Waindok et al., 2018). The published sequences were from 13 taxa of the genera *Corynosoma*, *Andracantha*, and *Pseudocorynosoma*. First of all, a ML phylogenetic tree was made by using a total of the 110 *cox1* sequences. The resultant phylogeny showed that the 97 isolates from Japan were divided into 6 clades. The topology of the clades was illustrated, using the representative isolates (Fig. 3). The mean values of the intra- and inter-clade pairwise divergence are shown in Table 3. All the values of the intra-clade divergence were at low levels (0.002 to 0.013), while those of the inter-clade divergence were significantly high, with the exception of a low value (0.027) between the clades 1 and 2. This low value seemed to be at an intraspecific level. Based on the comprehensive results of the DNA barcoding and morphological identification, the clades 1 and 2 were assigned to *C. strumosum*, the clade 3 to *C. semerme*, the clade 4 to *C. villosum*, the clade 5 to *Andracantha* sp., and the clade 6 to *A. phalacrocoracis*. *Corynosoma* sp. found from porpoises in Japan was included in the clade 2 of *C. strumosum*. A sequence of *Corynosoma magdaleni* (DNA database accession no. EF467872) (García-Varela and Pérez-Ponce de León, 2008) was treated as a junior synonym of *C. strumosum*.

A certain inconsistency was observed in the present phylogenetic tree, because the published sequences of *C. validum* (accession nos. JX442193 and MK119252) (García-Varela et al., 2013; Lisitsyna et al., 2019) were included in the clade 4 of *C. villosum*. Furthermore, the published sequence of *C. villosum* (accession no. MK119251) (Lisitsyna et al., 2019) were far different from those of the clade 4.

Another phylogenetic tree was made using nuclear 28S rDNA sequences (Supplementary Fig. 2). The phylogeny of the conservative sequences was mostly consistent with the classification of DNA barcoding. The mitochondrial clades 1 and 2 of *C. strumosum* were unseparated in the 28S rDNA tree, supporting the view of unifying the two clades.

The results of DNA barcoding of larval and adult acanthocephalans are summarized in Table 2. Out of 72 cystacanth isolates from fish, 66 (91.7%) were identified as one of three species of *Corynosoma*. The majority of them were *C. strumosum* and *C. villosum* from all the coastal seas of Hokkaido. The clade 2 of *C. strumosum* was found only from the coastal sea of the Pacific. There were no clear host-parasite relationships between fish species and *Corynosoma* species. All species of *Corynosoma* and *Andracantha* were found from the Arctic rainbow smelt (*Osmerus dentex*). However, flatfish as the starry flounder (*Pleuronectes stellatus*), the yellow striped flounder (*Pleuronectes herzensteini*), the Cresthead flounder (*Pleuronectes schrenki*), the northern black flounder (*Pleuronectes obscurus*), the dusky sole (*Pleuronectes mochigarei*), and the bastard halibut (*Paralichthys olivaceus*) frequently harbored *C. villosum*, a common species to cause human infections in Hokkaido.

3.3. Genetic variation of C. strumosum and C. villosum

The *cox1* genetic variations of *C. strumosum* and *C. villosum* were examined, using 18 isolates of the clade 1, 17 isolates of the clade 2, and 30 isolates of the clade 3 from fish. A parsimony haplotype network was drawn by each data set of the three clades, which was comprised of 1023 nucleotide sites. As shown in Fig. 4, an extensive sequence diversity was observed, particularly in the clades 1 and 3. Surprisingly,

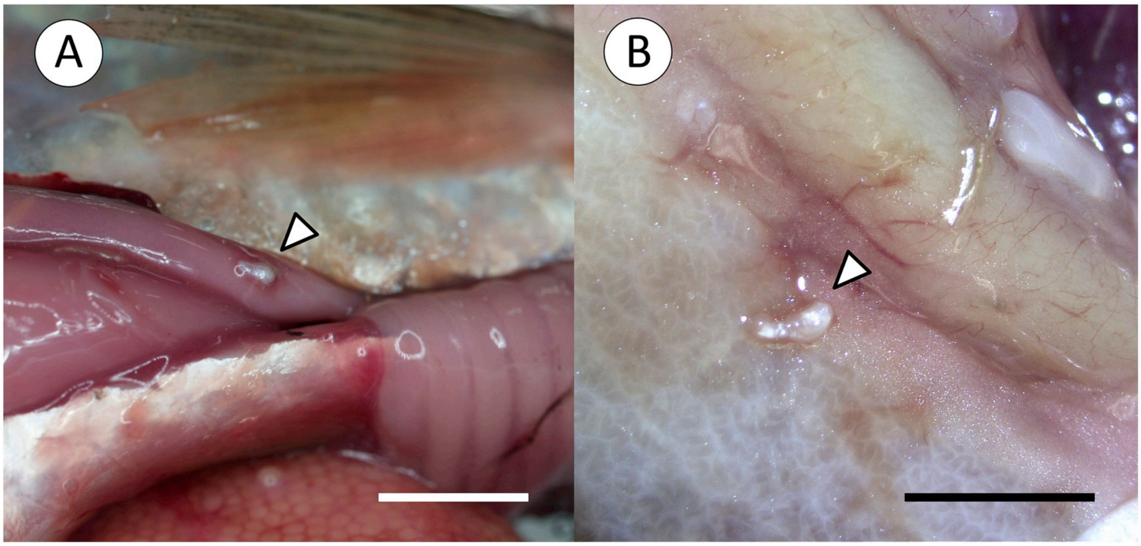

Fig. 2. Cystacanth larvae found from marine fish. A) The larva attached on the intestinal serosa of *Clupea pallasii*. B) Another larva on the intestinal serosa of *Osmerus dentex*. Scale bar 10 mm.

there were no common haplotypes in both the clades. The sea areas of host fish were completely unrelated to the network patterns of the clades 1 and 3, although haplotypes of the clade 2 were only found from the coastal sea of the Pacific. The population genetics indices of the three clades indicated that *C. strumosum* and *C. villosum* have kept mtDNA diversity without bottlenecking in the coastal seas of Hokkaido (Table 4).

3.4. Morphology of larval and adult acanthocephalans

Micrographs of cystacanth larvae recovered in this study are shown in Fig. 5A to E. All of the specimens were artificially evaginated with trypsin digestion. Here follows are brief description of the larvae.

Corynosoma strumosum (Fig. 5A). One male and four females were observed. The larva has an elongated trunk. The fore part of the dorsal area and the anterior half of the ventral area are covered with minute spines. The whole body (including fully extended proboscis) is 3.7 ± 0.4 mm long and 0.77 ± 0.06 mm wide. The hindtrunk is long and thin. The nearly cylindrical proboscis is 510 ± 29 μm long and 260 ± 22 μm wide, armed with 18 longitudinal rows consisting of 9–12 hooks per each row.

Corynosoma villosum (Fig. 5B). Two males and three females. The larva has a thick and short trunk. The fore part of the dorsal area and the anterior three-fourths of the ventral area are covered with minute spines. The whole body is 3.2 ± 0.2 mm long and 1.04 ± 0.06 mm wide. The foretrunk is obviously thick. The vase-shaped proboscis is 566 ± 36 μm long and 350 ± 34 μm wide, armed with 22–24 longitudinal rows consisting of 12–13 hooks per each row.

Corynosoma semerme (Fig. 5C). One female. The larva has a small trunk. The fore part of the dorsal area and all the ventral area covered with minute spines. The whole body is 2.7 mm long and 0.9 mm wide. The cylindrical proboscis is 571 μm long and 296 μm wide, armed with 22 longitudinal rows consisting of 11 hooks per each row.

Andracantha sp. (Fig. 5D). One female. The larva has a small elliptic trunk. The fore part of both the ventral and dorsal areas is covered with minute spines. The whole body is 2.4 mm long and 0.9 mm wide. The hindtrunk is very short. The proboscis is 650 μm long and 280 μm wide, armed with 16 longitudinal rows consisting of 9–10 hooks per each row. These features are similar to those of larval *Andracantha mergi* (Presswell et al., 2018; Schmidt, 1975).

Andracantha phalacrocoracis (Fig. 5E). One female. The larva has a long neck and a small elliptic trunk. The fore part of both the ventral and dorsal areas is covered with minute spines. The whole body is 2.8 mm long and 1.0 mm wide. The hindtrunk is very short. Proboscis is 460 μm long and 330 μm wide, armed with 18 longitudinal rows consisting of 11–12 hooks per each row.

The morphological characteristics of adult *Corynosoma* spp. from pinnipeds in Hokkaido were compared with those of the North American species (Van Cleave, 1953b) (Supplementary Table 1). There were almost no discrepancies in this comparison. The appearance of adult *C. villosum* in Hokkaido is shown in Fig. 5F. The morphological feature of this species was mutually consistent with the original description of *C. villosum* (Van Cleave, 1953a), although a serious inconsistency was observed in their DNA barcode sequences (Fig. 3).

Some of the specimens used in this study have been deposited as vouchers in the National Museum of Nature and Science, Tsukuba, Japan under the collection numbers NSMT-As 4604 to 4612.

4. Discussion

In this study, the infection status of different commercial fish species with cystacanth larvae was examined in all the coastal seas of Hokkaido to evaluate the infection risk of human corynosomiasis. The large-scale fish survey, based on the morphological and molecular identification of cystacanths, showed that 3 species of *Corynosoma* (*C. strumosum*, *C. villosum*, and *C. semerme*) and 2 species of *Androacantha* (*A. phalacrocoracis* and an unidentified species) prevail in commercial fish. Among them, *C. strumosum* and *C. villosum* are dominant species. Pinnipeds in Hokkaido consistently harbor only the 3 species of adult *Corynosoma* (H. Katahira and M. Kobayashi, unpublished data), supporting the occurrence of those larvae from marine fish. The species composition of *Corynosoma* in fish varies according to sea areas (Sinisalo and Valtonen, 2003). The comprehensive result of the present study suggests a high possibility that almost every commercial fish species in Hokkaido are infected with *C. strumosum* or *C. villosum* or both, irrespective of the fishery areas. However, the existence of uninfected fish species in the present data indicates that the cystacanth infections are fluctuated perhaps due to habitats and food habits of fish. The overall prevalence of cystacanth-infected fish was approximately 10% (Table 1), but the value is expected to be increased when examining hot spots around the colonies of pinnipeds. The present study also showed that flatfish frequently harbor *C. villosum*. However, the species identification data of cystacanths (Table 2) showed that each of *Corynosoma* spp. is a

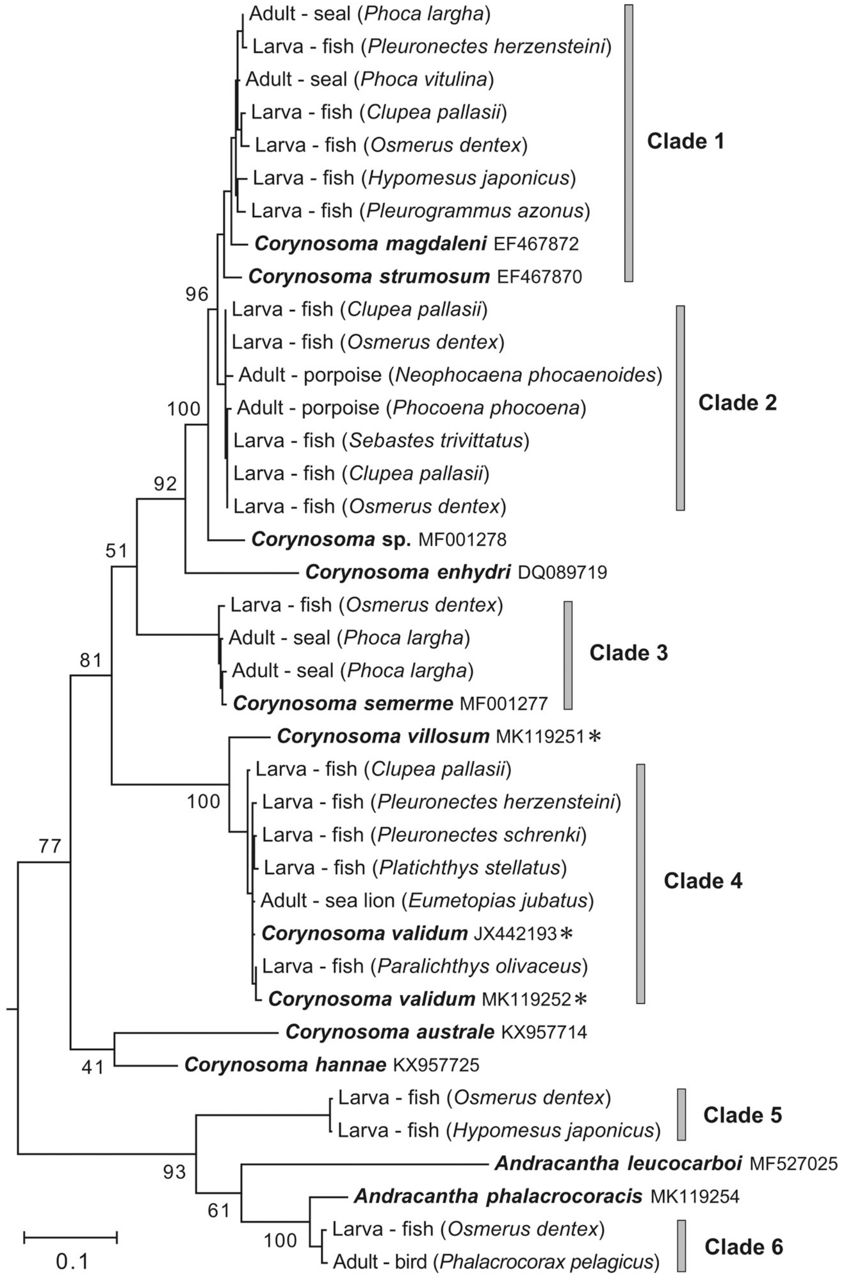

Fig. 3. A maximum likelihood phylogenetic tree showing evolutionary relationships among *Corynosoma* and related acanthocephalans from fish, birds, pinnipeds, and cetaceans in Hokkaido, Japan. The tree was constructed from sequences of mitochondrial *cox1* (575 nucleotide sites). Twenty-seven representative isolates were analyzed, together with 13 reference taxa from DNA databases. The nucleotide accession numbers were shown after the scientific name. The tree was rooted with an outgroup taxon, *Pseudocorynosoma anatarium* (accession no. KX688148). Adult worms from each clades were used as a standard for morphological identification of species. An asterisk denotes a conflict sequence to our morphological identification. Bootstrap percentages are shown on representative nodes. A scale bar indicates the number of substitutions per nucleotide site.

generalist in selecting fish as a paratenic host. A wide range of fish species should therefore be considered as the source of human infections.

The inshore and offshore fisheries are one of the most important industrial sectors in Hokkaido. The annual fisheries yield of Hokkaido reached to 1.27 million tons in 2014, which accounted for 27% of the total amount of Japan (Annual statistics on fishery and aquaculture production by Statistics Department, Ministry of Agriculture, Forestry and Fisheries). In the Japanese archipelago, pinnipeds are most common in the coastal seas of Hokkaido (Ohdachi et al., 2015). As a result of their distribution, marine fish of Hokkaido become infected with *Corynosoma* spp. Considering the wide range of commercial fish species of Hokkaido and the advanced transportation system of fresh fish (Katahira et al., 2017; Nagaishi et al., 2017), there is a possibility that human corynosomiasis will occur everywhere in Japan.

The Japanese custom of eating raw fish is responsible for various parasitic diseases due to protozoan and helminthic pathogens (Iwashita et al., 2013; Nawa et al., 2005). The location of the parasites in fish (e.g. scale, gills, serous membrane, and muscle) is very important in considering human infections. In this study, the encysted cystacanths of *Corynosoma* spp. were most frequently found on serous membrane covering the digestive tract of fish, but not in the muscle. As far as searching literatures to date, there are no reports on the cystacanths encysted within the muscle of fish. The internal organs of fish are usually not edible raw, and almost only the muscle is consumed uncooked (e.g. sushi and sashimi). Pickled foods made from the whole body of clupeid fish are unpopular in Japan. Accordingly, the risk of human infections with *Corynosoma* spp. appears to be virtually at low levels. In this study, we also noticed that the cystacanths were excysted in the peritoneal cavity of fish lacking in freshness. It is likely that the excysted or encysted cystacanths attached on the serosal lining of peritoneal muscle become the source of human infections. The water washing of fish after removing the guts, the elimination of the serous membrane of the peritoneal cavity, or the freezing of the whole body seems to be effective in preventing human corynosomiasis. The refrigerated storage of fish for several days is obviously ineffective in

Table 2
Species identification of acanthocephalans of the genera *Corynosoma* and *Andracantha* from fish, mammals, and birds in the coastal seas of Hokkaido by mitochondrial *cox1*-based DNA barcoding.

Host species	Coasts	Stages	Parasite species[a]	No. isolates identified
Osmerus dentex (fish)	Okhotsk	cystacanth	*C. strumosum* (clade 1)	1
	Pacific	cystacanth	*C. strumosum* (clade 1)	2
	Pacific	cystacanth	*C. strumosum* (clade 2)	14
	Pacific	cystacanth	*C. villosum* (clade 3)	1
	Pacific	cystacanth	*C. semerme* (clade 4)	1
	Pacific	cystacanth	*Andracantha* sp. (clade 5)	2
	Pacific	cystacanth	*A. pharacrocoracis* (clade 6)	3
Hypomesus japonicus (fish)	Pacific	cystacanth	*C. strumosum* (clade 1)	3
	Pacific	cystacanth	*Andracantha* sp. (clade 5)	1
Clupea pallasii (fish)	Okhotsk	cystacanth	*C. strumosum* (clade1)	3
	Okhotsk	cystacanth	*C. villosum* (clade 3)	7
	Pacific	cystacanth	*C. strumosus* (clade 2)	2
Pleurogrammus azonus (fish)	Okhotsk	cystacanth	*C. strumosus* (clade 1)	2
	Japan Sea	cystacanth	*C. villosum* (clade 3)	1
Gadus macrocephalus (fish)	Okhotsk	cystacanth	*C. villosum* (clade 3)	1
Platichthys stellatus (fish)	Okhotsk	cystacanth	*C. villosum* (clade 3)	5
Pleuronectes herzensteini (fish)	Pacific	cystacanth	*C. strumosum* (clade 1)	3
	Pacific	cystacanth	*C. villosum* (clade 3)	5
Pleuronectes schrenki (fish)	Okhotsk	cystacanth	*C. villosum* (clade 3)	1
Pleuronectes obscurus (fish)	Pacific	cystacanth	*C. strumosum* (clade 1)	1
	Pacific	cystacanth	*C. villosum* (clade 3)	3
Pleuronectes mochigarei (fish)	Japan Sea	cystacanth	*C. strumosum* (clade 1)	1
	Japan Sea	cystacanth	*C. villosum* (clade 3)	3
Paralichthys olivaceus (fish)	Japan Sea	cystacanth	*C. villosum* (clade 3)	2
Sebastes trivittatus (fish)	Pacific	cystacanth	*C. strumosum* (clade 2)	1
Myoxocephalus stelleri (fish)	Okhotsk	cystacanth	*C. strumosum* (clade 1)	2
	Okhotsk	cystacanth	*C. villosum* (clade 3)	1
Total (fish)			***C. strumosum* (clade 1)**	**18**
			***C. strumosum* (clade 2)**	**17**
			***C. villosum* (clade 3)**	**30**
			***C. semerme* (clade 4)**	**1**
			***Andracantha* sp. (clade 5)**	**3**
			***A. pharacrocoracis* (clade 6)**	**3**
			All species	**72**
Phoca largha (seal)	Okhotsk	adult	*C. strumosum* (clade 1)	4
	Okhotsk	adult	*C. semerme* (clade 4)	3
	Japan Sea	adult	*C. strumosum* (clade 1)	2
Phoca vitulina (seal)	Pacific	adult	*C. strumosum* (clade 1)	2
Eumetopias jubatus (sea lion)	Okhotsk	adult	*C. villosum* (clade 3)	4
Phocoena phocoena (porpoise)	Pacific	adult	*C. strumosum* (clade 2)	4
Neophocaena phocaenoides (porpoise)[b]	Pacific	adult	*C. strumosum* (clade 2)	2
Phalacrocorax pelagicus (cormorant)	Pacific	adult	*A. pharacrocoracis* (clade 6)	3
Phalacrocorax capillatus (cormorant)	Pacific	adult	*A. pharacrocoracis* (clade 6)	1
Total (mammals and birds)			**All species**	**25**
Ground total				**97**

[a] The mitochondrial phylogenetic clade of each species is shown in parentheses.
[b] The finless porpoise was originated from the Pacific coast of central Honshu (Kuramochi et al., 2000).

killing cystacanths, because this study confirmed that chilled fish in general markets kept cystacanths alive.

There are no clear ecological data on whether the population increase of pinnipeds has influence on the prevalence of fish infected with *Corynosoma*. Previous reports focused on the population dynamics of *Corynosoma* only in pinniped definitive hosts (Kaimoto et al., 2018; Sinisalo and Valtonen, 2003; Valtonen et al., 2004). The increase of Steller sea lions, harbor seals, and spotted seals in the coastal seas of Hokkaido has consequences for serious damages to fisheries (Haneda et al., 2017; Hoshino et al., 2006; Wilke, 1954). The recent findings of human corynosomiasis in Hokkaido might be indirectly related to the increase of the pinnipeds. Detailed ecological studies on the definitive, intermediate, and paratenic hosts of *Corynosoma* are required to elucidate the parasite transmission dynamics among them, and further to

Table 3
Intra- and inter-clade divergence of mitochondrial *cox1* sequences (1023 nucleotide sites) among the phylogenetic clades of *Corynosoma* and *Andracantha* from the coastal seas of Hokkaido.

Species[a]	Phylogenetic clade[b]	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5
C. strumosum (26)	Clade 1 (0.013)					
C. strumosum (23)	Clade 2 (0.004)	0.027				
C. villosum (34)	Clade 3 (0.007)	0.134	0.136			
C. semerme (4)	Clade 4 (0.007)	0.103	0.107	0.126		
Andracantha sp. (3)	Clade 5 (0.002)	0.178	0.174	0.175	0.177	
A. phalacrocoracis (7)	Clade 6 (0.013)	0.182	0.185	0.191	0.183	0.155

[a] A total number of both adult and larval isolates examined are shown in parentheses.
[b] The values of intra-clade divergence are shown in parentheses.

Table 4
Population genetics indices of mitochondrial *cox1* sequences (1023 nucleotide sites) of *Corynosoma* spp. from fishes in the coastal seas of Hokkaido.

Species	Clade	No. isolates examined	No. haplotypes	Haplotype diversity	Nucleotide diversity	Fu's FS	Tajima's D
C. strumosum	1	18	18	1.000	0.01252	−8.596	−1.58069
C. strumosum	2	17	15	0.985	0.00391	−10.638	−2.30084
C. villosum	3	30	30	1.000	0.00716	−29.969	−2.21310

apply to a science-based rational management of pinnipeds. The present population genetics data of larval *Corynosoma* from fish strongly suggest that the parasites are highly shuffled in the coastal seas of Hokkaido, probably due to the long-distance movements of pinnipeds and fish. Interestingly, the clade 2 of *C. strumosum* was restricted to fish in the Pacific Ocean, and its adult stage was found from porpoises. Furthermore, the genetic diversity of the clade 2 was relatively weak as compared to the clade 1, suggesting that the clade 2 is a new comer to Hokkaido. More parasite samples from porpoises are necessary to verify the hypothesis. The behavioral ecology of intermediate hosts is also important in considering the transmission of *Corynosoma*. Amphipod crustaceans involved as intermediate hosts are mostly detritivores or scavengers (Anderson and Bell, 2016). The frequent detection of *C. villosum* from flatfish living on the seafloor is probably related to the habitat preference of amphipods and their susceptibility to the parasites.

Morphological observations are important in the taxonomy of acanthocephalans. In the adult stage of *Corynosoma*, the whole appearance of trunk, the configuration of proboscis hooks, and the distribution of trunk spines are key characters to identify species. However, slight differences of the morphological characters cause a number of synonyms (Amin, 2013). A mitochondrial *cox1*-based DNA barcoding system is an essential tool of modern taxonomy to discriminate morphologically indistinguishable species and to link the different developmental stages of a species (Hebert and Gregory, 2005). The resultant molecular phylogeny is also useful in detecting synonyms. A recent taxonomic revision of *Corynosoma* regarded *C. obtuscens* as a junior synonym of *C. australe* by comparing their morphology and *cox1* sequences (Lisitsyna et al., 2019). Also in the present study, *C. magdaleni* was tentatively treated as a junior synonym of *C. strumosum*. The original description of *C. magdaleni* was based on the adult stage from grey seals (*Halichoerus grypus*) in Canada (Montreuil, 1958). A detailed morphological comparison between *C. strumosum* and *C. magdaleni* is needed for the conclusive decision of their specific status. The taxonomy of *Corynosoma* from cetaceans is also problematic. The present DNA barcoding system identified the large adults of *Corynosoma* sp. from *N. phocaenoides* and *P. phocoena* in Japan as the clade 2 of *C. strumosum*. *Corynosoma alaskensis* described from *P. phocoena* in Alaska (Golvan, 1959) is morphologically similar to our specimens. Further comparative studies are required to evaluate the validity of *C. alaskensis*.

The clinical samples of parasites from human patients are frequently formalin-fixed and then embedded in paraffin. A PCR-based DNA sequencing is applicable to the diagnosis of the paraffin-embedded specimen to a species level (Kaito et al., 2019; Koonmee et al., 2011; Tang et al., 2017). A previous clinical report from Hokkaido (Takahashi et al., 2016) tentatively identified the causative species of corynosomiasis as *C. validum* by sequencing the parasite *cox1* from the paraffin-embedded specimen. However, the present DNA barcoding system showed that the already published *cox1* sequence of the clinical isolate (DNA database accession no. LC077795) (Takahashi et al., 2016) should be included in *C. villosum*.

International DNA databases may contain erroneous sequences (*cox1*, 28S rDNA, and internal transcribed spacers of rDNA cassette) of *Corynosoma* spp., due to the misidentification of species. Some of them were marked as invalid (Lisitsyna et al., 2019). This state of confusion makes it difficult to conduct DNA barcoding. Therefore, in this study, the original sequences of morphologically identified adults of *Corynosoma* spp. were used as standards. The most problematic issue of this study is an inconsistency of DNA barcode (*cox1*) sequences between *C. villosum* and *C. validum*. The morphological discrimination of *C. villosum* from *C. validum* is easy, because the hindtrunk of male and female *C. villosum* is spineless in the ventral side, whereas in female *C. validum* the ventral spines reach to the posterior end. A cylindrical proboscis is also unique to *C. validum* (Van Cleave, 1953b). Therefore, the possibility of misidentification of *C. villosum* can be denied in this study. There are two possible reasons for the inconsistency of the *cox1* sequences; one is a human error in the published sequences (García-Varela et al., 2013; Lisitsyna et al., 2019), the other is that an ancient mitochondrial introgression occurred in the Far Eastern population of *C. villosum*. A direct comparison between the Far Eastern and North American populations of *C. villosum* is required to resolve the inconsistency of the *cox1* sequences.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108256.

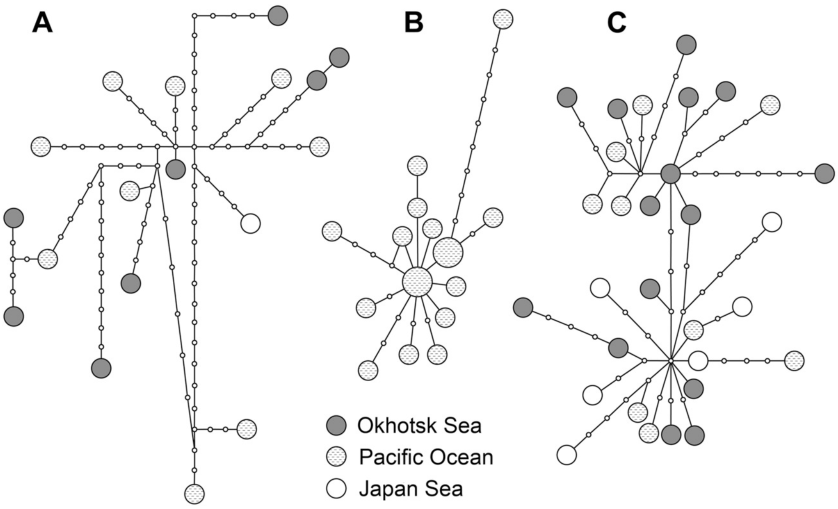

Fig. 4. Statistical parsimony networks of *Corynosoma strumosum* and *Corynosoma villosum*. The networks were made from sequences of mitochondrial *cox1* (1023 nucleotide sites). All larval isolates from fish were subjected to the analysis. The size of circles indicates the frequency of haplotypes; however, most of the circles contain only one isolate because of a strong diversity within populations. The circles are classified into three types due to the sea areas of Hokkaido. Hypothetical haplotypes are shown as small circles. A) The network of the clade 1 of *C. strumosum* ($n = 18$). B) The network of the clade 2 of *C. strumosum* ($n = 17$). C) The network of *C. villosum* ($n = 30$).

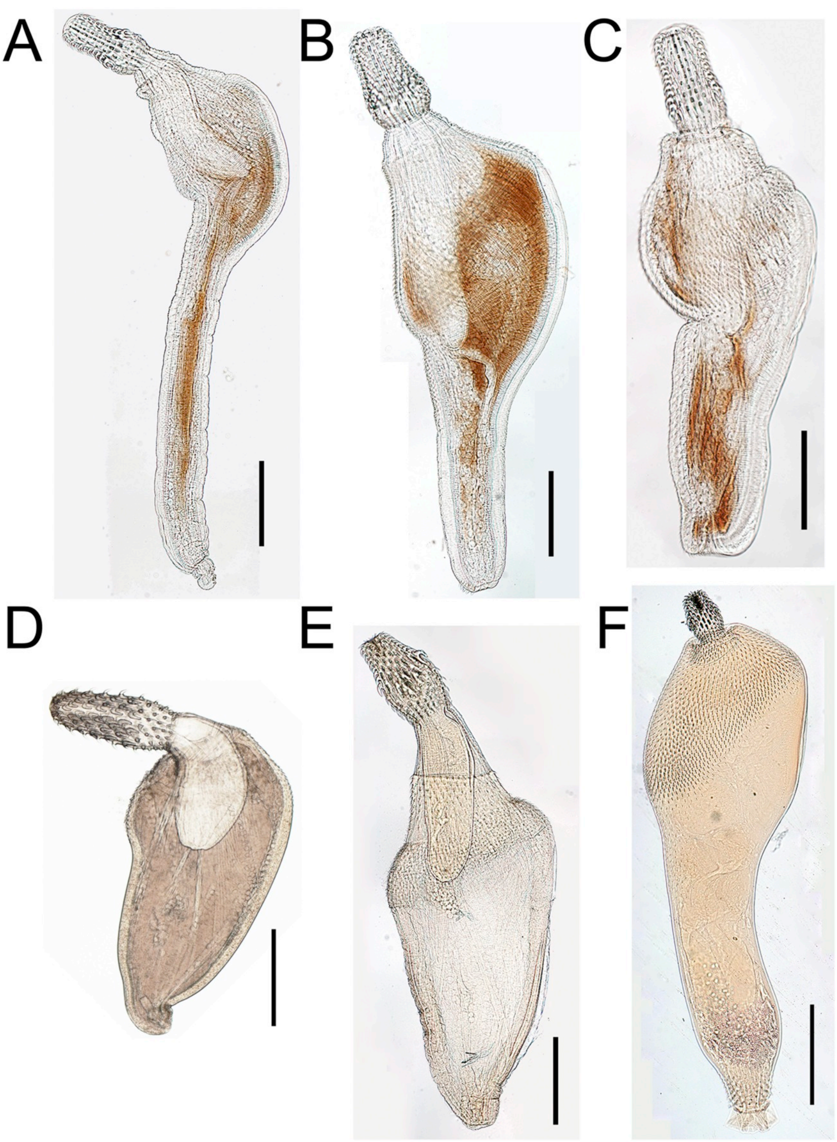

Fig. 5. Micrographs of acanthocephalans found in this study. Scale bars represent 500 μm. A) Cystacanth of *Corynosoma strumosum* from the herring (*Clupea pallasii*) in Yubetsu. B) Cystacanth of *Corynosoma villosum* from the yellow striped flounder (*Pleuronectes herzensteini*) in Rausu. C) Cystacanth of *Corynosoma semerme* from the Arctic rainbow smelt (*Osmerus dentex*) in Nemuro. D) Cystacanth of *Andracantha* sp. from the Japanese surf smelt (*Hypomesus japonicus*) in Nemuro. E) Cystacanth of *Andracantha phalacrocoracis* from *O. dentex* in Nemuro. D) Adult of *Corynosoma villosum* from the Steller sea lion (*Eumetopias jubatus*) in Abashiri. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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References

Amin, O.M., 2013. Classification of the acanthocephala. Folia Parasitol. 60, 273–305.

Anderson, G.S., Bell, L.S., 2016. Impact of marine submergence and season on faunal colonization and decomposition of pig carcasses in the Salish Sea. PLoS One 11, e0149107.

Araki, J., Machida, M., 1987. Some acanthocephalans from marine fish of northern Japan, with descriptions of two new species, *Acanthocephaloides ichiharai* and *A. claviformis*. Bull. Natl. Sci. Mus. Tokyo Ser. A. 13, 1–11.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.

Crompton, D.W.T., Nickol, B.B., 1985. Biology of the Acanthocephala. Cambridge University Press, pp. 285–305.

Delyamure, S.L., 1968. Helminthfauna of marine mammals: Ecology and phylogeny. In: Israel Program for Scientific Translations, pp. 339–352.

Fujita, T., 1921. On the parasites of Japanese fish II. Zool. Mag. 33, 1–8.

Fujita, T., Waga, E., Kitaoka, K., Imagawa, T., Komatsu, Y., Takanashi, K., Anbo, F., Anbo, T., Katuki, S., Ichihara, S., Fujimori, S., Yamasaki, H., Morishima, Y., Sugiyama, H., Katahira, H., 2016. Human infection by acanthocephalan parasites belonging to the genus *Corynosoma* found from small bowel endoscopy. Parasitol. Int. 65, 491–493.

García-Varela, M., Nadler, S.A., 2006. Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. Mol. Phylogenet. Evol. 40, 61–72.

García-Varela, M., Pérez-Ponce de León, G., 2008. Validating the systematic position of *Profilicollis* Meyer, 1931 and *Hexaglandula* Petrochenko, 1950 (Acanthocephala: Polymorphidae) using cytochrome *c* oxidase (Cox 1). J. Parasitol. 94, 212–217.

García-Varela, M., Pérez-Ponce de León, G., Aznar, F.J., Nadler, S.A., 2013. Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. Mol. Phylogenet. Evol. 68, 176–184.

García-Varela, M., Henández-Orts, J.S., Pinacho-Pinacho, C.D., 2017. A morphological and molecular study of *Pseudocorynosoma* Aznar, Pérez Ponce de León and Raga 2006 (Acanthocephala: Polymorphidae) from Mexico with the description of a new species and the presence of *cox* 1 pseudogenes. Parasitol. Int. 66, 27–36.

Golvan, Y.J., 1959. Acanthocéphales du genre *Corynosoma* Lühe 1904, parasites de Mammifères d'Alaska et de Midway. Ann. Parasitol. Hum. Comp. 34, 288–321.

Haneda, T., Kobayashi, M., Tamura, Z., Takada, K., Ogawa, I., 2017. Coastal use patterns of Japanese harbor seals (*Phoca vitulina stejnegeri*) in Akkeshi Bay in Hokkaido during the spring season. Mamm. Sci. 57, 35–43.

Hebert, P.D., Gregory, T.R., 2005. The promise of DNA barcoding for taxonomy. Syst. Biol. 54, 852–859.

Hernández-Orts, J.S., Smales, L.R., Pinacho-Pinacho, C.D., García-Varela, M., Presswell, B., 2017. Novel morphological and molecular data for *Corynosoma hannae* Zdzitowiecki, 1984 (Acanthocephala: Polymorphidae) from teleosts, fish-eating birds and pinnipeds from New Zealand. Parasitol. Int. 66, 905–916.

Hoshino, H., Isono, T., Takayama, T., Ishinazaka, T., Wada, A., Sakurai, Y., 2006. Distribution of the Steller sea lion *Eumetopias jubatus* during winter in the northern sea of Japan, along the west coast of Hokkaido, based on aerial and land sighting surveys. Fish. Sci. 72, 922–931.

Ito, M., Sato, T., Shirai, W., Kikuchi, S., 1998. Parasites and related pathological lesions in the gastrointestinal tract of a seal (*Phoka vitulina* Linnaeus). J. Vet. Med. Sci. 60, 1025–1028.

Iwashita, Y., Kamijo, Y., Nakahashi, S., Shindo, A., Yokoyama, K., Yamamoto, A., Omori, Y., Ishikura, K., Fujioka, M., Hatada, T., Takeda, T., Maruyama, K., Imai, H., 2013. Food poisoning associated with *Kudoa septempunctata*. J. Emerg. Med. 44, 943–945.

Kaimoto, T., Hirazawa, T., Masubuchi, T., Morohoshi, A., Katahira, H., Kobayashi, M., 2018. Host characteristics and infection level of an intestinal parasite *Corynosoma strumosum* (Acanthocephala) in the Kuril harbor seal of Erimo Cape, Hokkaido, Japan. Parasitol. Int. 67, 237–244.

Kaito, S., Sasaki, M., Goto, K., Matsusue, R., Koyama, H., Nakao, M., Hasegawa, H., 2019. A case of small bowel obstruction due to infection with *Bolbosoma* sp. (Acanthocephala: Polymorphidae). Parasitol. Int. 68, 14–16.

Katahira, H., Fujita, T., Nakao, M., Haneda, T., Kobayashi, M., 2017. Corynosomiasis: a minor parasitic zoonosis originated from pinniped mammals as final hosts. Mamm. Sci. 57, 361–365.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kenyon, K.W., Wilke, F., 1953. Migration of the northern fur seal, *Callorhinus ursinus*. J. Mammal. 34, 86–98.

Kobayashi, M., Kouno, Y., Ito, M., Nishina, M., Fujimoto, Y., Kato, K., 2009. Seasonal change in number and movement pattern of spotted seals (*Phoca largha*) migrating around the sea of Japan. PICES Sci. Rep. 36, 76–81.

Kobayashi, Y., Tatsuya, K., Chishima, J., Fujii, K., Wada, K., Baba, S., Itoo, T., Nakaoka, T., Kawashima, M., Saito, S., Aoki, N., Hayama, S., Osa, Y., Osada, H., Niizuma, A., Suzuki, M., Uekane, Y., Hayashi, K., Kobayashi, M., Ohtaishi, N., Sakurai, Y., 2014. Population trends of the Kuril harbor seal *Phoca vitulina stejnegeri* from 1974 to 2010 in southeastern Hokkaido, Japan. Endanger. Species Res. 24, 61–72.

Koonmee, S., Intapan, P.M., Yamasaki, H., Sugiyama, H., Muto, M., Kuramochi, T., Kularbkeaw, J., Kanpittaya, J., Maleewong, W., Nawa, Y., 2011. Molecular identification of a causative parasite species using formalin-fixed paraffin embedded (FFPE) tissues of a complicated human pulmonary sparganosis case without decisive clinical diagnosis. Parasitol. Int. 60, 460–464.

Kovacs, K.M., Aguilar, A., Aurioles, D., Burkanov, V., Campagna, C., Gales, N., Gelatt, T., Goldsworthy, S.D., Goodman, S.J., Hofmeyr, G.J.G., Härkönen, T., Lowry, L., Lydersen, C., Schipper, J., Sipilä, T., Southwell, C., Stuart, S., Thompson, D., Trillmich, F., 2011. Global threats to pinnipeds. Mar. Mamm. Sci. 28, 414–436.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Kuramochi, T., Kikuchi, T., Okamura, H., Tatsukawa, T., Doi, H., Nakamura, K., Yamada, T., Koda, Y., Yoshida, Y., Matsuura, M., Sakakibara, S., 2000. Parasitic helminth and epizoit fauna of finless porpoise in the inland sea of Japan and the Western North Pacific with a preliminary note on faunal difference by host's local population. Mem. Natn. Sci. Mus., Tokyo 33, 83–95.

Lisitsyna, O.I., Kudlai, O., Spraker, T.R., Tkach, V.V., Smales, L.R., Kuzmina, T.A., 2019. Morphological and molecular evidence for synonymy of *Corynosoma obtuscens* Lincicome, 1943 with *Corynosoma australe* Johnston, 1937 (Acanthocephala: Polymorphidae). Syst. Parasitol. 96, 95–110.

Lühe, M., 1911. Acanthocephalen. In: Brauer, A. (Ed.), Süsswasserfauna Deutschlands. Eine Exkursionfauna 16. Gustav Fischer, pp. 35–40.

Machida, M., 1966. Helminths collected from a pelagic shag. Bull. Nat. Sci. Mus. Tokyo 9, 447–451.

Machida, M., 1969. Parasites of the northern fur seal and their relationship to the breeding islands. Proc. Jap. Soc. Syst. Zool 5, 16–17.

Mizuno, M., Sasaki, T., Kobayashi, M., Haneda, T., Masubuchi, T., 2018. Mitochondrial DNA reveals secondary contact in Japanese harbour seals, the southernmost population in the western Pacific. PLoS One 13, e0191329.

Montreuil, P., 1958. *Corynosoma magdaleni* sp. nov. (acanthocephala), a parasite of the grey seal in eastern Canada. Can. J. Zool. 36, 205–215.

Nagaishi, H., Inada, T., Yoshioka, T., Sato, A., 2017. Development of a compact, onboard slurry icemaker to rapidly produce optimal ice for maintaining freshness of marine products. Synthesiol. 10, 1–10.

Nagasawa, K., 1999. Parasites of pinnipeds (Mammalia: Carnivora) in Japan: checklist and bibliography. Bull. Nat. Res. Inst. Far Seas Fish 36, 27–32.

Nakabo, T., 2013. Fishes of Japan with Pictorial Keys to the Species, Third edition. Tokyo University Press, Hadano (xlix + 2428 pp).

Nakamura, S., Mikami, N., Takahashi, H., 1965. Some helminthes from seals. JAZA. 7, 35–38.

Nawa, Y., Hatz, C., Blum, J., 2005. Sushi delights and parasites: the risk of fishborne and foodborne parasitic zoonoses in Asia. Clin. Infect. Dis. 41, 1297–1303.

Nickol, B.B., Helle, E., Valtonen, E.T., 2002. Corynosoma magdaleni in gray seals from the Gulf of Bothnia, with emended descriptions of Corynosoma strumosum and Corynosoma magdaleni. J. Parasitol. 88, 1222–1229.

Ohdachi, S.D., Ishibashi, Y., Iwasa, M.A., Fukui, D., Saitoh, T. (Eds.), 2015. The Wild Mammals of Japan, Second edition. Shoukadoh Book Sellers and the Mammal Society of Japan, Kyoto, pp. 272–277.

Presswell, B., García-Varela, M., Smales, L.R., 2018. Morphological and molecular characterization of two new species of *Andracantha* (Acanthocephala: Polymorphidae) from New Zealand shags (Phalacrocoracidae) and penguins (Spheniscidae) with a key to the species. J. Helminthol. 92, 740–751.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34, 3299–3302.

Schmidt, G.D., 1971. Acanthocephalan infection of man, with two new records. J. Parasitol. 57, 582–584.

Schmidt, G.D., 1975. *Andracantha*, a new genus of Acanthocephala (Polymorphidae) from fish-eating birds, with descriptions of three species. J. Parasitol. 61, 615–620.

Shiozaki, A., Amano, M., 2017. Population- and growth-related differences in helminthic fauna of finless porpoises (*Neophocaena asiaeorientalis*) in five Japanese populations. J. Vet. Med. Sci. 79, 534–541.

Sinisalo, T., Valtonen, E.T., 2003. *Corynosoma* acanthocephalans in their paratenic fish hosts in the northern Baltic Sea. Parasite 10, 227–233.

Takahashi, K., Ito, T., Sato, T., Goto, M., Kawamoto, T., Fujinaga, A., Yanagawa, N., Saito, Y., Nakao, M., Hasegawa, H., Fujiya, M., 2016. Infection with fully mature *Corynosoma* cf. *validum* causes ulcers in the human small intestine. Clin. J. Gastroenterol. 9, 114–117.

Tang, T.H., Wong, S.S., Lai, C.K., Poon, R.W., Chan, H.S., Wu, T.C., Cheung, Y.F., Poon, T.L., Tsang, Y.P., Tang, W.L., Wu, A.K., 2017. Molecular identification of *Spirometra erinaceieuropaei* tapeworm in cases of human sparganosis, Hong Kong. Emerg. Infect. Dis. 23, 665–668.

Valtonen, E.T., Helle, E., Poulin, R., 2004. Stability of *Corynosoma* populations with fluctuating population densities of the seal definitive host. Parasitology 129, 635–642.

Van Cleave, H.J., 1953a. A preliminary analysis of the acanthocephalan genus Corynosoma in mammals of North America. J. Parasitol. 39, 1–13.

Van Cleave, H.J., 1953b. Acanthocephala of northern American mammals. Illinois Biological Monographs 23, 1–179.

Waindok, P., Lehnert, K., Siebert, U., Pawliczka, I., Strube, C., 2018. Prevalence and molecular characterisation of Acanthocephala in pinnipedia of the north and Baltic seas. Int. J. Parasitol. Parasites Wildl. 7, 34–43.

Wakazono, T., 1953. Some parasites in the intestine of a seal, *Phoca vitulina* Linneus, 1758. Mem. Res. Fac. Agric. Hokkaido Univ. 1, 312–314.

Wilke, F., 1954. Seals of northern Hokkaido. J. Mammal. 35, 218–224.

Yagi, K., Ohfuchi, M., Uraguchi, K., Kijima, T., 2002. *Corynosoma villosum* (Acanthocephala: Polymorphidae) recovered from the human colon. Report of the Hokkiado Institute of Public Health 52, 134.

Yamaguti, S., 1963. Systema Helminthum. In: Acanthocephala. vol. V John Wiley & Sons, Inc., London (423 pp).