

Occurrence of Anisakid Nematodes in Walleye Pollock (*Gadus chalcogrammus*) Caught off the East Sea of Korea: Their Molecular Identification and Biological Implication

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Abstract – Walleye pollock (*Gadus chalcogrammus*) is one of the popular fish species in Korea and known to frequently harbor anisakid nematodes. Despite many papers regarding walleye pollock having been published, there is no information on the prevalence and distribution of anisakid nematodes from walleye pollock in Korean waters. We investigated 716 walleye pollock caught from Goseong, the East Sea of the Korean Peninsula during May 2015–December 2016. In total, 1,085 nematodes were collected and subsequently identified by PCR-RFLP analysis of internal transcribed spacer region of ribosomal DNA. The prevalence and mean intensity of anisakid nematodes was 63.6% (68/107) and 4.4 larvae/fish (297/68) in samples of 2015, and 21.5% (131/609) and 6.0 larvae/fish (788/131) in samples of 2016. The most abundantly isolated anisakid nematode was *Anisakis simplex* (Rudolphi, 1809) (73.5%, 798/1,085), followed by *Contracaecum osculatum* (Rudolphi, 1802) (3.1%, 34/1,085), *Hysterothylacium aduncum* (Rudolphi, 1802) (2.9%, 31/1,085), *Anisakis pegreffii* (Campana-Rouget and Biocca, 1955) (1.4%, 15/1,085), and a hybrid genotype (0.4%, 4/1,085). The mitochondrial DNA *cox2* gene sequences of randomly selected *A. simplex* showed the highest similarity (99.5%–98.8%) with those from chub mackerel (*Scomber japonicus*) in Japan or from chum salmon (*Oncorhynchus keta*) in Korea. The mtDNA *cox2* gene sequences of *C. osculatum* showed the highest similarity (99.0%) with those from bearded seals in Japan. All of these results give us clues to the geographical distribution, migration route and prey items of walleye pollock caught off Korea. Further extensive analyses will be necessary to get more information on the biology of Korean walleye pollock.

Keywords – *Gadus chalcogrammus*, walleye pollock, *Anisakis simplex*, *Contracaecum osculatum*, PCR-RFLP

1. Introduction

Anisakid nematodes have been widely reported to infect marine vertebrates around the world and many different species of hosts are known to be involved in their life cycles; marine crustaceans (euphausiids and mysids) play a role as the intermediate hosts, fish (teleost) and squids (cephalopods) as the transport/paratenic hosts, and cetaceans (dolphin, whales) and pinniped (seals, sea lions, and walrus) as the definitive/final host (Mattiucci and Nascetti 2008). As the transport/paratenic hosts, fish prey marine crustaceans infected by third stage larvae (L3) of anisakid nematodes. Many studies revealed that diverse fish species are infected with anisakid nematodes and play a role as their transport/paratenic hosts. In particular, commercially important fish species such as European hake *Merluccius merluccius*, spotted mackerel *Scomber australasicus*, Pacific cod *Gadus macrocephalus* have been well-studied and known to have diverse species of anisakid nematodes (Abe et al. 2005; Chen and Shih 2015; Cipriani et al. 2015; Jeon et al. 2016).

The larvae of anisakid nematodes infect not only marine vertebrates, but also accidentally infect humans through the ingestion of raw or undercooked fish or squids (Cipriani et al. 2015; Quiazon et al. 2011). Although larval anisakids cannot molt further to adults, they can enter the gastrointestinal tracts of humans and cause problems such as anisakiasis, characterized by epigastric pain, nausea, and vomiting (Audicana and Kennedy 2008). Moreover, the anisakid nematodes have many allergens causing clinical symptoms ranging from urticaria, gastrointestinal and/or respiratory signs and anaphylaxis (Baird et al. 2014). Some of these allergens are heat-stable,

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and thus can be problematic even in well-cooked seafood (Alonso-Gomez et al. 2004; Quiazon et al. 2009). In recent decades, many researchers have reported thousands of cases of anisakiasis around the world, and most of the cases are known to occur in Japan with 2000–3000 cases reported annually (Chai et al. 2005).

Clear identification of anisakid nematodes is essential for accurate diagnosis and epidemiological studies. Conventional identification of anisakid nematodes has been conducted by morphological observation. But, this approach lacks information on diagnostic features at the species level (Mattiucci and Nascetti 2008), and the specimens need to be in good condition to provide clear results. Molecular techniques, such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the internal transcribed spacer (ITS) region, direct sequencing of ribosomal DNA (rDNA), and mitochondrial DNA cytochrome oxidase subunit 2 (mtDNA *cox2*) can overcome these shortcomings and have been widely used for the species identification of the anisakid nematodes (Kim et al. 2016; Mattiucci and Nascetti 2008).

Walleye pollock (*Gadus chalcogrammus*) inhabit cold and deep water columns, being regarded as one of the most important fish species due to its abundance in the northern North Pacific Ocean (Kang and Kim 2015; NFRDI 2010; Nishimura and Yamada 1988). In the Bering Sea, yields of walleye pollock reach one million tonnes per year, amounting to one billion dollars (Holtcamp 2010). However, in Korea, the annual catch of walleye pollock has continuously decreased since 1985, with its complete disappearance occurring in the 2000s. Many factors, such as global warming and overfishing are thought to be involved in the decrease of the walleye pollock population (Kang and Kim 2015).

Young walleye pollock feed on krill, zooplankton, and other crustaceans. As they grow, their diet begins to include juvenile pollock and other teleosts (Yamamura et al. 2002). All of these are known to be the transport/paratenic host of anisakid nematodes (Mattiucci and Nascetti 2008). Given that Korean people have enjoyed almost all parts of walleye pollock with various methods of cooking, they can be exposed to the risk of experiencing anisakiasis and thus it is important to properly identify the nematodes found in walleye pollock. Several authors have examined the infection of walleye pollock by anisakid nematodes (Quiazon et al. 2009; Umehara et al. 2006), but they identified the anisakid nematodes with only a small number of fish samples, thus providing insufficient information on the anisakid nematode

fauna of walleye pollock. In this study, we investigated anisakid nematodes from walleye pollock caught near Ayajin Port, Goseong, facing the East Sea of Korea on a monthly basis during 2015–2016. The collected nematodes were identified by PCR-RFLP and amplification of the mtDNA *cox2* gene with subsequent sequencing, to provide information on the anisakid nematode fauna of walleye pollock and discuss the epidemiology of anisakid nematodes of walleye pollock in Korea.

2. Materials and Methods

Sample collection

In total, 716 walleye pollock were collected by bottom or midwater trawls during May 2015–December 2016. One hundred seven individuals were sampled in 2015 and 609 were sampled in 2016, respectively. Sampling location and biological information of fish samples are summarized in Fig. 1 and Table 1. The fish samples were immediately frozen after collection, transported to the laboratory and thawed at room temperature or kept at -20°C until examination. All of the fish were measured and weighed individually and carefully examined for nematodes in the viscera after opening the body cavity. The muscles were sliced and placed between transparent glass plates, pressed and observed under the light for existence of nematodes. The collected nematodes were washed repeatedly with 0.9% NaCl solution and preserved individually in absolute alcohol for molecular analysis. Prevalence (the number of infected host/the number of examined host), mean intensity of anisakid nematodes (the average number of a particular parasite species in a single infected host), and abundance (the number of collected nematodes/the number of examined host) were calculated as quantitative descriptors of the parasite population as previously described by Bush et al. (1997).

DNA extraction and PCR

All of the collected nematodes were analyzed for molecular identification. Each larva was washed three times with PBS and placed in 2.0 ml Eppendorf tube containing 400 μl Holmes and Bonner lysis buffer (100 mM Tris-HCl pH 8.0; 7 M urea; 10 mM EDTA pH 8.0; 350 mM NaCl; 2% SDS) and incubated overnight at 60°C after adding 5 μl Proteinase K (Biolabs Inc. New England). Genomic DNA was extracted using the Phenol-chloroform method with a slight modification (Wasko et al. 2003). The DNA pellet was resuspended in 100 μl

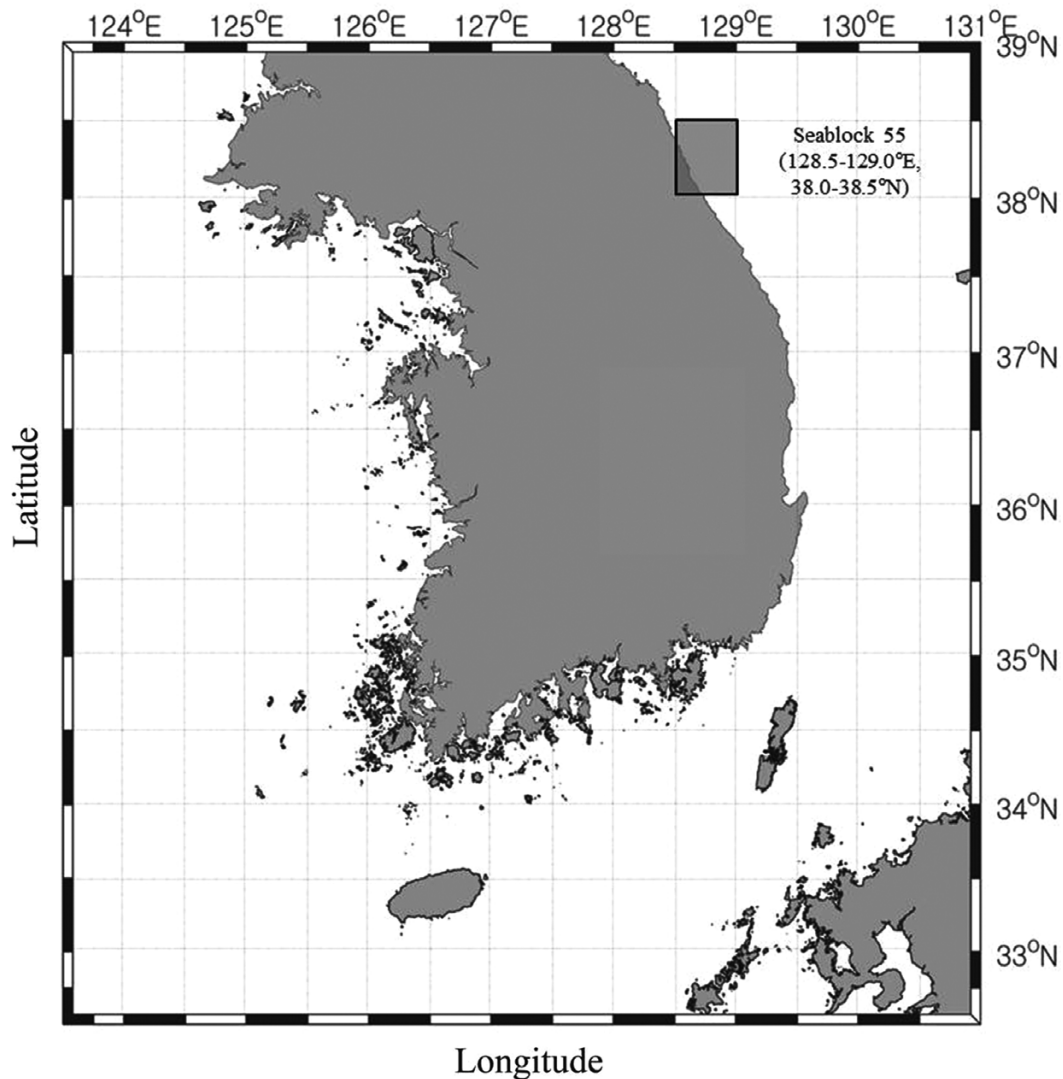


Fig. 1. Sampling locations of walleye pollock (*Gadus chalcogrammus*) in this study

Table 1. Measurement data of walleye pollock caught off Ayajin Port, Korea in this study

Sampling year	Number of fish	BW ^a (g) (mean $\pm$ SD ^b)	BL ^c (cm) (mean $\pm$ SD)
2015	107	467.1 $\pm$ 177.9	43.1 $\pm$ 4.0
2016	609	265.5 $\pm$ 217.5	33.1 $\pm$ 8.0
Total	716	332.7 $\pm$ 204.3	36.4 $\pm$ 6.7

^aBW, body weight; ^bSD, standard deviation; ^cBL, body length

UltraPureTM DNase/RNase-free distilled water (Invitrogen, USA). DNA concentration and purity were measured using Nanodrop 1000 (Thermo Scientific, USA). The ITS region (ITS1-5.8S-ITS2) of rDNA was amplified using primers A (forward: 5'-GTCGAATTCGTAGGTGAACCTGCGGAAGGATCA-3') and B (reverse: 5'-GCCGGATCCGAATCCTGGTTA GTTTCTTTTCCT-3') (D'Amelio et al. 2000). PCR was conducted using 2 μ l extracted DNA as a template, 1 μ l for

each primer in total volume of 20 μ l AccuPower[®] PCR Premix (Bioneer, Korea), containing 1U Taq DNA polymerase, 250 μ M dNTPs (dATP, dCTP, dGTP, dTTP), 10mM Tris-HCl (pH 9.0), 30 mM KCl and 1.5 mM MgCl₂. Amplification was conducted using MyCyclerTM (BioRad, USA), with the following condition: denaturation at 94°C for 5 min, then 35 cycles at 94°C for 40 s, 54°C for 40 s, 72°C for 90 s, and post amplification at 72°C for 7 min.

RFLP analysis

The PCR products were digested with restriction enzymes *Hinf*I, *Rsa*I, *Hha*I, and *Taq*I (D'Amelio et al. 2000). The digestion was performed using 3.5 µl of PCR products, 0.5 µl of each restriction enzyme, 1 µl of CutSmart® buffer (New England Biolabs, USA) and distilled water up to a final volume of 10 µl. The digestion with these enzymes was conducted at 37°C for 90 min. The digested products were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide (Sigma, USA). The pictures were taken using Gel Logic 100 Imaging System (Biostep, Germany) supported by Kodak Molecular Imaging System Software 4.0.

Mitochondrial DNA *cox2* gene amplification and sequencing

Mitochondrial DNA *cox2* gene was amplified using primers 211 (forward: 5'-TTTCTAGTTATATAGATTGRTTYAT-3') and 210 (reverse: 5'-CACCAACTCTTAAATTATC-3') (Nadler and Hudspeth 2000). PCR conditions are as follows: initial denaturation at 94°C for 3 min, 34 cycles were run at 94°C for 30 s, 46°C for 1 min, and 72°C for 90 s followed by post-amplification step at 72°C for 10 min. PCR products were purified by *AccuPrep*® Gel Purification Kit (Bioneer, Korea) according to the manufacturer's instruction. 10 ng/µl of purified PCR products were directly sequenced by ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, USA).

Phylogenetic analysis

The sequence of mtDNA *cox2* region was aligned with sequence references from GenBank (NCBI) using Clustal W method (Thompson et al. 1994). To compare the genetic relationship among other published sequences, the phylogenetic tree was constructed by using MEGA software version 6 (Tamura et al. 2013). The obtained nucleotide sequences were registered in GenBank.

Statistical analysis

A spearman's rank test was applied to explore the relationship

between abundance and body length of fish samples with 95% confidence interval. Statistical analysis was performed with SPSS 21.0 software (IBM, Chicago, IL).

3. Results

Infection levels

In total, 27.8% of fish (199/716) were infected by anisakid nematodes. The prevalence and mean intensity of anisakid nematodes was 63.6% (68/107) and 4.4 (297/68) in samples of 2015, and 21.5% (131/609) and 6.0 (788/131) in samples of 2016 (Table 2). When the prevalence was analyzed for the host body length class, walleye pollock with more than 20.0 cm body length were found to be infected with the nematodes in 2016, while those with more than 25.0 cm body length were infected with the nematodes in 2015 (Fig. 2). Although the sampling period and the number of sampled fish were not the same between 2015 and 2016, the abundance of nematodes increased with the body length of fish. Host body length was significantly positively correlated with the abundance of anisakid nematodes ($r = 0.223$, $p < 0.05$ for samples in 2015; $r = 0.372$, $p < 0.01$ for samples in 2016) (Fig. 3).

Most of the nematodes were found encysted (coiled in a flat spiral) in the liver (74.7%, 811/1,085) or existed freely in the body cavities and caecum (16.0%, 174/1,085). The rest of nematodes were found in the intestine (6.2%, 67/1,085) both inside and on the surface of the tract. The rest of them were found in the stomach (2.6%, 28/1,085) and mesentery (0.5%, 5/1,085). The information on infection levels are shown in Table 2.

Identification of nematodes by PCR-RFLP

The amplification of ITS fragment (spanning ITS-1, the 5.8S subunit, and ITS2) from all samples produced 1 kb in length (data not shown). Amplicons were subsequently digested using *Hinf*I, *Rsa*I, *Hha*I, and *Taq*I restriction enzymes. This

Table 2. Infection levels of nematodes in walleye pollock

Sampling year	Prevalence (%) ^a	Mean intensity of anisakid nematodes ^b	% of anisakid nematodes (number of anisakid nematodes in each body organ/total number of anisakid nematodes)				
			Liver	Body cavity	Intestine	Mesentery	Stomach
2015	63.6 (68/107)	4.4 (297/68)	77.1 (229/297)	9.8 (29/297)	7.4 (22/297)	0.7 (2/297)	5.1 (15/297)
2016	21.5 (131/609)	6.0 (788/131)	73.9 (582/788)	18.4 (145/788)	5.7 (45/788)	0.4 (3/788)	1.6 (13/788)
Total	27.8 (199/716)	5.5 (1,085/199)	74.7 (811/1,085)	16.0 (174/1,085)	6.2 (67/1,085)	0.5 (5/1,085)	2.6 (28/1,085)

^aPrevalence: the number of infected host/the number of examined host

^bMean intensity of anisakid nematodes: the average number of anisakid nematodes in a single infected host

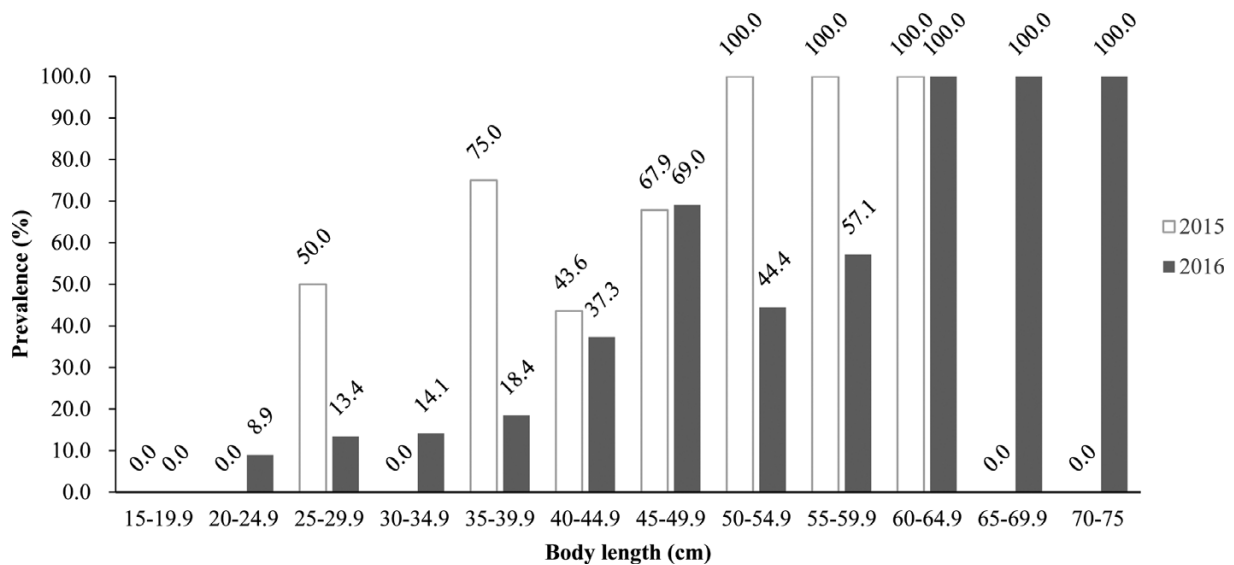


Fig. 2. Prevalence of anisakid nematodes infection in walleye pollock (*Gadus chalcogrammus*) based on host body length

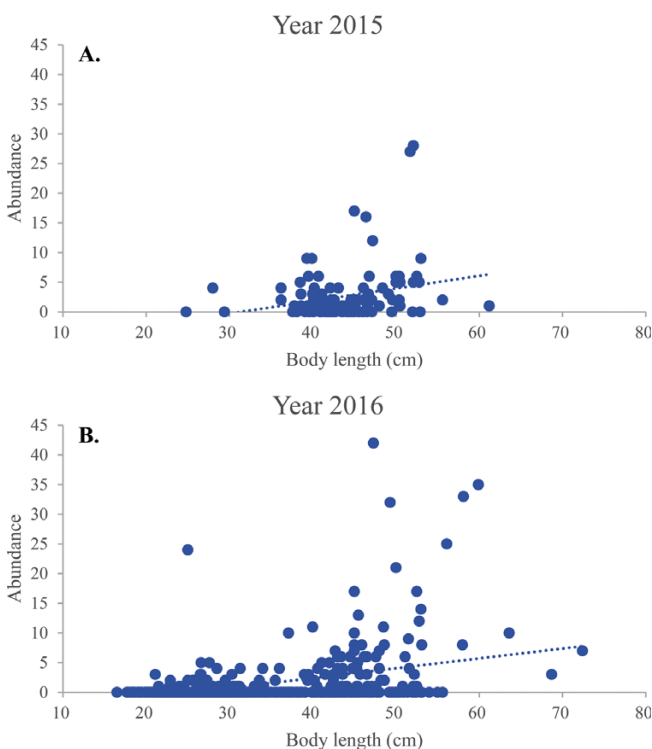


Fig. 3. Relationships between abundance of anisakid nematode larvae and body length of walleye pollock

approach yielded distinct banding patterns belonging to the genus *Anisakis*, *Contracaecum*, and *Hysterothylacium*. Digestion by *Hinf*I showed 5 different patterns, i.e., 620-250 bp, 350-300-250 bp, 620-350-300-250 bp, 500-480 bp, and 700-350 bp, respectively (Fig. 4A). Digestion by *Rsa*I displayed 3

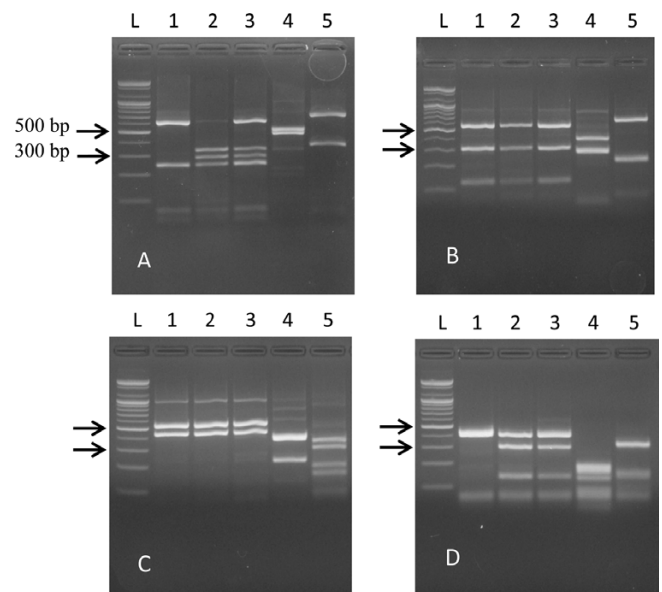


Fig. 4. PCR-RFLP profiles of the nematode species obtained by digestion of PCR products with *Hinf*I (A), *Rsa*I (B), *Hha*I (C), and *Taq*I (D) restriction enzymes, respectively. (L: ladder, 1: *A. simplex*, 2: *A. pegreffii*, 3: hybrid genotype, 4: *C. osculatum*, 5: *H. aduncum*)

different patterns, i.e., 550-300 bp, 400-300 bp, and 650-220 bp (Fig. 4B). Digestion by *Hha*I also showed 3 different patterns, i.e., 550-430 bp, 380-220 bp, and 350-300-200-150 bp respectively (Fig. 4C). Digestion by *Taq*I showed 4 different patterns, i.e., 430-400 bp, 400-300 bp, 200-180 bp, and 350 bp (Fig. 4D). The banding patterns observed in this study matched the known patterns of *Anisakis simplex* (lane 1, Fig.

4A–D), *Anisakis pegreffii* (lane 2, Fig. 4A–D), hybrid genotype (*A. simplex* x *A. pegreffii*) (lane 3, Fig. 4A–D), *Contracaecum osculatum* (lane 4, Fig. 4A–D), and *Hysterothylacium aduncum* (lane 5, Fig. 4A–D), respectively.

The most frequently identified nematodes in this study were *A. simplex* (73.5%, 798/1,085), followed by *C. osculatum* (3.1%, 34/1,085), *H. aduncum* (2.9%, 31/1,085), *A. pegreffii* (1.4%, 15/1,085), and a hybrid genotype (0.4%, 4/1,085). The remaining 203 samples (18.7%) could not be subjected to the PCR amplification process due to the unexpected failure of the DNA extraction procedure. The results for anisakid nematodes assemblage in walleye pollock are summarized in Table 3.

The anisakid nematode species assemblage in walleye pollock was expressed as cumulative bars, depending on the host body length (Fig. 5). In general, walleye pollock were infected with various species of anisakid nematodes. The

most frequently found nematode, *A. simplex* was found in all size classes of walleye pollock and although the occurrence fluctuated, it generally increased with host body length. In particular, walleye pollock longer than 55 cm in 2015 and 60 cm in 2016 had only *A. simplex*.

Sequencing and phylogenetic analysis

Because of the huge number of samples found in this study, 10 samples identified as *A. simplex* and 5 samples identified as *C. osculatum* by PCR-RFLP were randomly selected and sequenced for mtDNA cox2 gene. Amplification of mtDNA cox2 gene generated 629 bp size products (data not shown). The phylogenetic tree was constructed with mtDNA cox2 gene sequences of these samples and those of samples previously registered in NCBI. The mtDNA cox2 gene sequences of *A. simplex* (Genbank accession number: MF964205–MF964214) were clustered with those isolated

Table 3. Anisakid nematodes assemblage of walleye pollock in this study

Organ	% of anisakid nematodes (number of anisakid nematodes in each body organ/total number of anisakid nematodes)					
	<i>A. simplex</i>	<i>C. osculatum</i>	<i>H. aduncum</i>	<i>A. pegreffii</i>	Hybrid genotype	Failed
Liver	82.8 (671/811)	0.4 (3/811)	1.4 (11/811)	1.4 (11/811)	0.5 (4/811)	13.6 (111/811)
Body cavity	58.9 (103/174)	15.4 (27/174)	1.1 (2/174)	1.1 (2/174)	0.0 (0/174)	23.4 (40/174)
Intestine	22.4 (15/67)	4.5 (3/67)	13.4 (9/67)	1.5 (1/67)	0.0 (0/67)	58.2 (39/67)
Stomach	21.4 (6/28)	0.0 (0/28)	32.1 (9/28)	0.0 (0/28)	0.0 (0/28)	46.4 (13/28)
Mesentery	60.0 (3/5)	20.0 (1/5)	0.0 (0/5)	20.0 (1/5)	0.0 (0/5)	0.0 (0/5)
Total	73.5 (798/1,085)	3.1 (34/1,085)	2.9 (31/1,085)	1.4 (15/1,085)	0.4 (4/1,085)	18.7 (203/1,085)

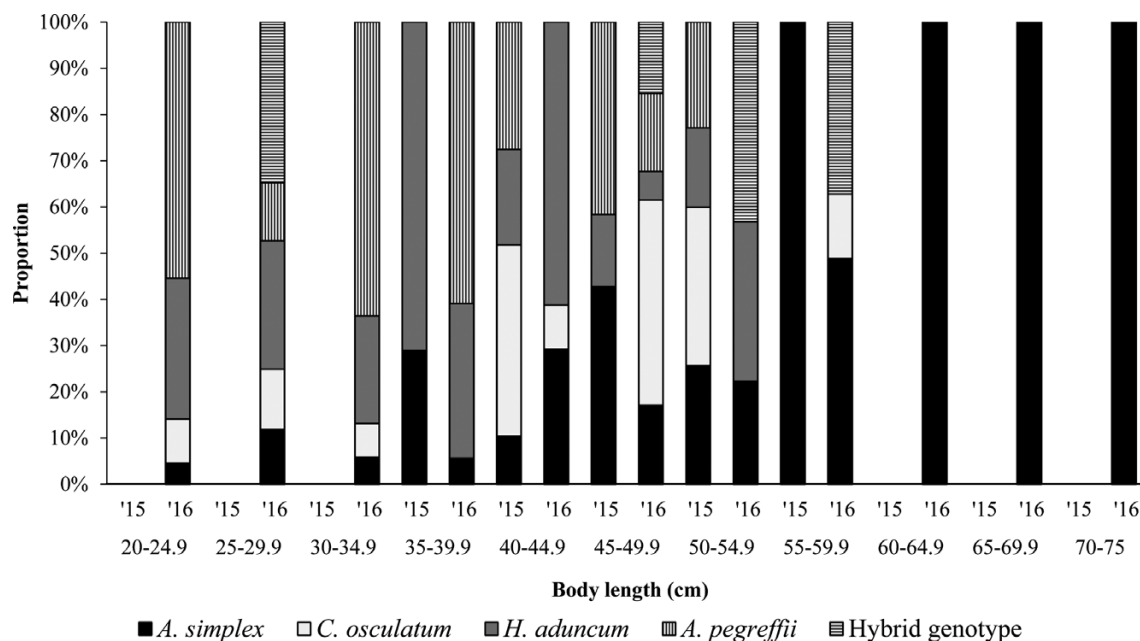


Fig. 5. The proportion of anisakid nematodes species in different body size group of walleye pollock

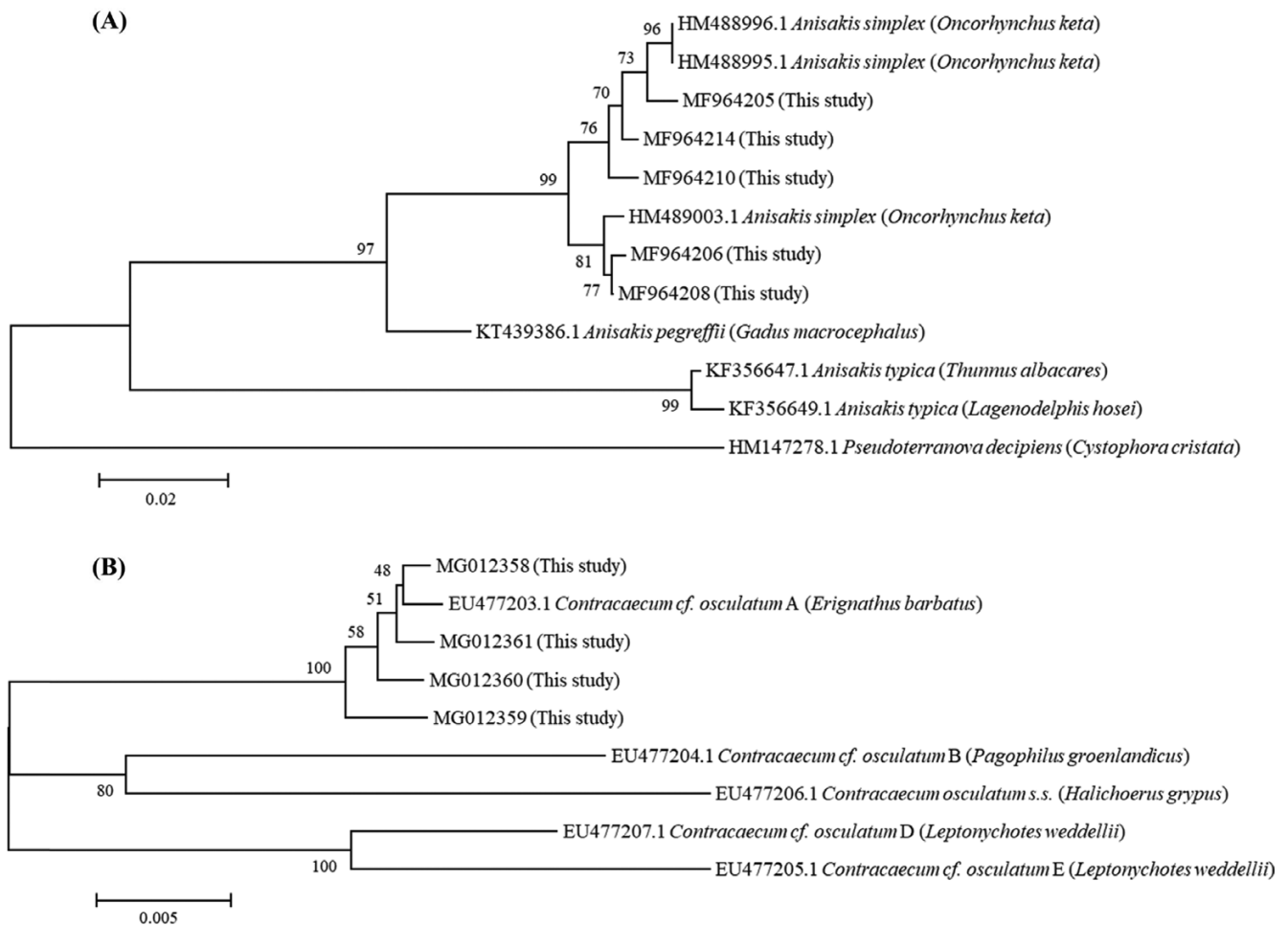


Fig. 6. Molecular phylogenetic tree showing the genetic relationships among *Anisakis simplex* (A) and *Contracaecum osculatum* (B) based on mtDNA *cox2* gene sequences. Scientific names of host for each nematode species are stated in parentheses. Analysis was performed using software MEGA version 6 and the scale bar indicates evolutionary distances

from chum salmon (*Oncorhynchus keta*) in South Korea and chub mackerel (*Scomber japonicus*) in Japan (Fig. 6A), with 98–99% similarities. The mtDNA *cox2* gene sequences of *C. osculatum* (Genbank accession number: MG012357–MG012361) were clustered with those isolated from bearded seals (*Erignathus barbatus*) in Japan (Fig. 6B), with 99% similarities.

4. Discussion

Walleye pollock has been known as the paratenic host of anisakid nematodes as well as other gadoid fish such as Pacific cod (*Gadus macrocephalus*), Atlantic cod (*Gadus morhua*), European hake (*Merluccius merluccius*), and blue whiting (*Micromesistius poutassou*) (Abe et al. 2005; Cipriani et al. 2015; Stromnes and Andersen 1998; Valero et al. 2000).

The results of the present study also confirmed the presence of anisakid nematodes in walleye pollock from Korea.

When the infection data of the 2 sampling years were statistically analyzed in relation with the host body length, the positive correlation between the larva abundance and the host body length was found both in 2015 and 2016 (Fig. 3). In general, the abundance of anisakid nematodes is known to have a positive relationship with the length of fish; many previous studies have shown that the prevalence of anisakid nematodes infection increased linearly with the body length in many fish species such as hake, anchovies, and chub mackerel (Abattouy et al. 2011; Mladineo 2003; Rello et al. 2009). The positive correlation between the length of the host and the number of larvae is thought to be attributed to the cumulative effect of repeated parasite infections, acquired over a longer lifetime for larger fish and constant reinfection

through the diet (Mladineo and Poljak 2014). In addition, we could observe anisakid larvae in walleye pollock longer than 20 cm (Fig. 2). This is also probably because of the cumulative effect of repeated infection in larger fish. The number of anisakid larvae in smaller fish (less than 25 cm in 2015 and 20 cm in 2016) is thought to be relatively low in our samples, and thus they might be difficult to detect with the naked eye.

In this study, most of the nematodes were found embedded in the liver (74.7%) or from body cavities of the walleye pollock (16.0%) and the rest of the nematodes were found in the viscera (Table 2). The nematodes found in the viscera were spread both inside and on the surface of the intestines, stomach and mesentery. The high infection level of anisakid nematodes in the liver was also recorded in hake (64.5%) and whiting (63.7%) (Mladineo and Poljak 2014). Since the liver is bigger than the other visceral organs and enriched with lipids, anisakid larvae are thought to easily burrow into and feed on liver tissue (Cheng 1976). In addition, gadiform fish are non-fatty species in which lipids are stored mainly in the liver or mesenteries (Smith 1984; Smith and Wootton 1975). All of this can explain why most of the larvae were found in the liver in our study. In addition, it is thought that consumers in Korea rarely come across live nematodes in walleye pollock because all of the fish available in markets are either fully frozen or dried products.

Many studies have reported that gadoid fish are a suitable intermediate/paratenic host for anisakid nematodes; for example, Pacific cod in Korea is known to be infected mainly with *A. pegreffii* (75.6%) (Jeon et al. 2016). However, of 1,085 nematodes from walleye pollock molecularly analyzed by PCR-RFLP, most of them were identified as *A. simplex* (73.5%) in this study. Even though these 2 fish species belong to the same gadoid family, they showed different anisakid nematodes fauna.

For *A. simplex*, their geographical distribution is known to be widespread between 30°N and the Arctic Circle (Mattiucci and Nascetti 2008). One of their transport/paratenic host, walleye pollock, are semi-demersal species residing in cold and deep water along the Asian and North American coasts, with the East Sea of Korean Peninsula acting as the southern boundary of their distribution (Kang and Kim 2015). And their final host, balaeopterid and delphinid cetaceans, are also distributed around the Korean peninsula (Sohn et al. 2012). Therefore, *A. simplex* are thought to complete their life cycle with these host, explaining the dominant occurrence

of this anisakid nematode species in walleye pollock of Korea. A similar host-parasite relationship was also observed in chum salmon *Oncorhynchus keta* from Korea (Setyobudi et al. 2011). The sequences of mtDNA *cox2* gene of randomly selected *A. simplex* revealed 98.8% of the homology with those from chum salmon (Fig. 6A), reflecting the overlapping of geographical distribution or migration route of 2 different fish species. For *A. pegreffii*, they are widely distributed between 30°N and 55°S (Mattiucci and Nascetti 2008). One of their transport/paratenic host, Pacific cod, is distributed in the North Pacific Ocean from the Yellow Sea to California Coast, along the Bering Strait (Bakkala et al. 1984). And their final host, delphinid cetaceans, are also distributed around the Korean Peninsula. However, the populations of Pacific cod in Korea are thought to be genetically different from those in other countries such as Japan, Russia, and USA (Kim et al. 2010), which means there may be no significant chance for Korean Pacific cod to be infected with *A. simplex*. Therefore, *A. pegreffii* are thought to complete their life cycle with these hosts in rather warm marine environments and are dominantly found in Korean Pacific cod.

In our study, *C. osculatum* was found and molecularly characterized for the first time in Korea. Genus *Contracaecum* belongs to the family Anisakidae and has a complex life cycle, involving many host species; of 5 known species, *C. osculatum* (Rudolphi, 1802) Baylis, 1937 mature in stomachs of pinniped and fish-eating birds as definitive hosts and release eggs into seawater with the feces. Hatched larvae are then transported by crustaceans to fish and squids as paratenic hosts and finally transmitted again to pinnipeds such as bearded seal *Erignathus barbatus*, grey seal *Halichoerus grypus* and fish-eating birds such as brown pelican *Pelecanus occidentalis*, black cormorant *Phalacrocorax carbo* as the definitive host (Nascetti et al. 1993; Mattiucci et al. 2008; Mattiucci and Nascetti 2008). Genus *Contracaecum* is also known to have several biological species that are morphologically very similar, but reproductively isolated, as in the case of genus *Anisakis* (Mattiucci and Nascetti 2008). Three siblings species (*C. osculatum* A, *C. osculatum* B, and *C. osculatum* C) are known to be spread among Atlantic and Arctic-Boreal regions, and the 2 species (*C. osculatum* D and *C. osculatum* E) are known to occur in the Antarctic region (Nascetti et al. 1993; Orecchia et al. 1994). The present study showed light infection by *C. osculatum* of walleye pollock (3.1%, Table 3). Since pinniped species such as bearded seals and Japanese sea lions which can act as the definitive host for *C. osculatum*

are known to be extinct around the Korean Peninsula (Heath and Perrin 2008), the infection of walleye pollock by *C. osculatum* caught off the East Sea of Korea might have occurred because of the existence of pinniped species in other areas. The phylogenetic tree constructed based on the mtDNA cox2 sequences demonstrated that the *C. osculatum* isolated from Korean pollock and from bearded seal from Japan were located in the same cluster (Fig. 6B), suggesting the geographical distribution of walleye pollock overlaps with that of pinniped species, as in the case of *A. simplex* discussed above.

Dynamic associations among fish host size, diet and parasites have been described (Münster et al. 2015). Anisakid nematodes have marine planktonic or semi-planktonic crustaceans as their first intermediate hosts and fish as intermediate or paratenic hosts. The first intermediate hosts can be eaten by a wide variety of transport or paratenic hosts including cephalopods and fishes, and many different life cycle patterns at this stage may occur, mostly depending on the different nematode species (Mattiucci and Nascetti 2008). The genus *Anisakis* is considered to perform a pelagic life cycle, while the genus *Contracaecum* is considered to perform benthic and pelagic life cycles (Busch et al. 2012). Given that walleye pollock show a broad food spectrum, depending on life history stage as well as body size and habitat (Yamamura et al. 2002), the different anisakid nematode assemblage in different body size class of host fish observed in this study can be explained, but clear conclusions await further study because we did not conduct stomach content analysis of host fish and the number of individual fish in each body size class were not even.

Parasites can provide a reliable guide to understand the biology of their host fish such as host diet, feeding behavior, migration, nursery ground, recruitment patterns, life history, stock discrimination, phylogenies and so forth (Catalano et al. 2014). This information has made parasites useful biological tags for marine fish and in fact, there have been many papers regarding anisakid nematodes as biological tags for studying marine fish stocks (Mattiucci et al. 2015 and the references therein). Our data is also thought to be useful for understanding the biology of walleye pollock as discussed above and further studies will be also indispensable to assess walleye pollock stock structure in Korea.

In summary, our study shows that walleye pollock are mainly infected by *A. simplex* and less frequently with *C. osculatum*. A small number of *A. pegreffii*, a hybrid genotype,

and *H. aduncum* were also discovered. *A. pegreffii* has been described as dominantly occurring in many marine fish species from Korean waters (Bak et al. 2014; Jeon et al. 2016; Kim et al. 2016; Lee et al. 2009). However, in this study, we found a different assemblage of anisakid nematode in walleye pollock having *A. simplex* as the dominant species. These phenomena indicate that the geographical distribution of different anisakid nematode species is closely related with the geographical distribution(s) of their successive hosts at different trophic levels, and thus their life-history stages are deeply embedded in aquatic food webs (Mattiucci and Nascetti 2008).

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