Occurrence of anisakid nematode larvae in chub mackerel (Scomber japonicus) caught off Korea

Tae-Jong Bak, Chan-Hyeok Jeon, Jeong-Ho Kim

Department of Marine Bioscience, Gwangju-Wonju National University, Gwangju 210-702, Republic of Korea

A R T I C L E   I N F O

Article history:
Received 24 April 2014
Received in revised form 29 July 2014
Accepted 6 September 2014
Available online 18 September 2014

Keywords:
Anisakis pegreffii
Anisakis simplex
Hysterothylacium sp
Chub mackerel
Scomber japonicus

Chemical compounds studied in this article:
Tris–HCl (PubChem CID: 93573)
dATP (PubChem CID: 15993)
dCTP (PubChem CID: 65091)
dGTP (PubChem CID: 65103)
dTTP (PubChem CID: 64968)
Potassium Chloride (PubChem CID: 4873)
Magnesium Chloride (PubChem CID: 24584)
EDTA (PubChem CID: 6050)
SDS (PubChem CID: 342365)
Urea (PubChem CID: 1176)

A B S T R A C T

Chub mackerel (Scomber japonicus) is a pelagic fish species widely distributed in the Indo-Pacific and a commercially important fish species in Korea. It is known to harbor anisakid nematodes larvae, and ingesting the raw or undercooked fish can accidentally cause human infection. In this study, we isolated the nematode larvae in 417 chub mackerel caught from 7 sampling locations around the Korean Peninsula in 2011 and 2012, and identified them by PCR-RFLP of the ITS (internal transcribed spacer) of ribosomal DNA and the direct sequencing of the mitochondrial DNA cox2 gene. The prevalence of infection was 55.4% (231/417) and the mean intensity was 7.0 (1628/231). Most of the nematodes (1523/1628; 93.6%) were found in the body cavity, while 5.5% (89/1628) were found in the gastrointestinal tract. Four different species were identified by PCR-RFLP and direct sequencing. Most of the nematodes (1535/1628; 94.3%) were identified as Anisakis pegreffii, and 2.8% (46/1628) were identified as Hysterothylacium sp. A hybrid genotype (Anisakis simplex sensu stricto × A. pegreffii) and A. simplex sensu stricto were 2.5% (41/1628) and 0.4% (6/1628) of the identified nematodes, respectively. The anisakid nematode assemblage of chub mackerel in Korea was similar to that of chub mackerel from the Tsushima Current stock in Japan, in that A. pegreffii was the dominant species. Since most of the anisakid nematodes were found in the body cavity and most of them were identified as A. pegreffii or Hysterothylacium sp. by PCR-RFLP and direct sequencing, chub mackerel may not greatly contribute to human anisakidosis in Korea. Alternatively, A. pegreffii may be responsible for human anisakidosis in Korea, in addition to A. simplex sensu stricto. Further studies, such as the molecular diagnosis of human anisakidosis, are necessary for assessing the epidemiological role of chub mackerel in Korea.

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1. Introduction

Genus Anisakis (Family Anisakidae) nematodes are parasites of the alimentary tract of aquatic vertebrates with worldwide distribution (Mattucci and Nascetti, 2008). Their life cycles are completed in aquatic ecosystems, and involve various hosts at different levels in food webs. Many teleosts and cephalopods are known to serve as intermediate or paratenic hosts for the nematodes, including commercially important species such as herring (Clupea spp.), cod (Gadus spp.), salmon (Oncorhynchus spp.), mackerel (Scomber spp.) and anchovy (Engraulis spp.) (Audicana and Kennedy, 2008).

The accidental ingestion of their third stage larvae (L3) in humans can cause gastrointestinal problems known as anisakidosis, and allergic reactions (Audicana and Kennedy, 2008). Anisakidosis frequently occurs in countries where the custom of eating raw or undercooked fish is common, particularly Japan and Korea (Kim et al., 2006a; Umehara et al., 2007). However, it has recently been increasing worldwide, probably due to the increased consumption of raw or undercooked fish and improved diagnostic capabilities (Audicana and Kennedy, 2008). Furthermore, these nematodes can be seen in fish flesh and viscera with the naked eye, giving a negative impression to consumers.

Anisakid nematodes L3 have been reported from various fish species and most of them have been conventionally diagnosed as Anisakis simplex, the most widespread species, by morphological observation (Audicana and Kennedy, 2008). However, morphological features have limitations for the identification of Anisakis larvae at the species level because there are few morphological characters of taxonomic significance in this group (Mattucci and Nascetti, 2008). Biochemical
and molecular techniques can overcome this limitation and have been used to demonstrate that there are 9 nominal *Anisakis* species in 2 phylogenetic clades. For example, the morphospecies *A. simplex* complex consists of 3 sibling species, *A. simplex* sensu stricto (s.s.), *A. pegreffii* and *A. simplex* C, according to PCR-RFLP and direct sequencing of the ITS (ITS-1 and ITS-2) and the 5.8 subunit rRNA gene or the cox2 gene of mitochondrial DNA (mt DNA) (Mattiucci and Nascetti, 2008; Umehara et al., 2006).

Chub mackerel (*Scomber japonicus*) is a commercially important species in East Asia, comprising 10–25% of the total annual marine fish catch of Korea (Hwang et al., 2008). It is generally consumed boiled or roasted, but also frequently as a raw dish. Particularly in Japan, it has been commonly consumed raw and has been confirmed as a main cause of human *Anisakis* infection (Umehara et al., 2007). Although chub mackerel in Korea is also known to be infected with anisakid nematodes (Kang et al., 2008; Lee et al., 2009), information on the anisakid nematode fauna of chub mackerel in Korea is still fragmentary because identification has only been conducted with a small number of fish lacking geographical information in their studies. Moreover, no epidemiological survey of human anisakidosis, based on a molecular approach has been conducted to date, and it remains somewhat unclear which fish species is the most frequently involved in human outbreaks in Korea.

The aim of the present study was to identify anisakid nematodes at the species level in chub mackerel caught from several sampling locations around the Korean Peninsula in 2011–2012. All the collected anisakid nematodes were genetically characterized by PCR-RFLP of the rDNA region comprising the ITS and the selective sequencing of the mtDNA cox2 gene. The diversity of anisakid nematodes in chub mackerel from different locations around the Korean Peninsula was assessed, and the possible relationships between the anisakid nematode assemblages and the occurrence of anisakidosis in Korea were discussed.

### 2. Materials and methods

#### 2.1. Fish samples

Chub mackerel were caught by seine nets during 2011–2012 at 7 sampling locations around the Korean Peninsula: 4 locations to the east, 2 locations to the south, and 1 location to the west. Information on sampling locations, dates, and fish samples are summarized in Fig. 1 and Table 1. Fish were immediately frozen or packed with ice once caught and transported to the laboratory. Fresh specimen were measured, dissected, and carefully examined for nematodes. Frozen specimens were defrosted, measured, and dissected for nematodes. The prevalence (P, the number of infected hosts/the number of examined hosts) and mean intensity (MI, the number of parasites/the number of infected hosts) of the nematodes were calculated for the quantitative description of the parasite population, as previously described (Bush et al., 1997).

#### 2.2. DNA extraction and PCR-RFLP

Isolated nematodes were carefully washed with PBS 3 times and individually placed in sterilized 1.5 ml Eppendorf tubes with 500 μL PBS. Genomic DNA was then extracted by the method of Wasko et al. (2003), with some modifications. Briefly, each larva was homogenized...
with 500 μL TNEs-digestion buffer (10 mM Tris–HCl pH 8.0; 125 mM NaCl, 10 mM EDTA pH 8.0; 0.5% SDS; 4 M urea), incubated with 1 μL RNase (Bioneer, Korea) at 42 °C for 1 h, and incubated at 60 °C overnight after adding 5 μL Proteinase K (Bioneer, Korea). Then, the same volume of phenol chlorform isoamyl alcohol (Bioneer, Korea) was added to the mixture and incubated for 15 min. After centrifugation, the supernatant was discarded and the pellet was resuspended with 80 μL of NE buffer (10 mM Tris–HCl pH 8.0; 1 mM EDTA pH 8.0). The concentration and purity of DNA were measured by a Nanodrop 1000 (Thermo Scientific, USA).

The PCR was conducted using 2 μL of the extracted DNA as a template in a total volume of 20 μL AccuPower® PCR PreMix (Bioneer, Korea) containing 1U Taq DNA polymerase, 250 μM dNTPs (dATP, dCTP, dGTP, dTTP), 10 mM Tris–HCl, 1.5 mM MgCl2. The ITS region of rDNA was amplified using primers 210 (forward: 5′-GTCGAACTCTGATTGAACCTGGGAGGACTCA-3′) and B (reverse: 5′-GCCGATCCATCTGGATGTGTTTCTTCTTCTT-3′) (D’Amelio et al., 2000). Amplification was conducted using MyCycler™ (Bio-Rad, USA), under the following conditions: denaturation at 94 °C for 10 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 was conducted using the restriction enzymes HinfI, Rsal, and HaeIII (D’Amelio et al., 2000; Umehara et al., 2006). Five μL of PCR product, 1 μL of each restriction enzyme, and 2 μL of NE buffer were mixed, and distilled water was added up to a final volume of 20 μL. The digestion was performed at 37 °C. All the reactions were conducted for 60 min and 10 μL of each digested product was analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide and visualized under UV light.

### 2.3. Mitochondrial DNA cox2 gene amplification and phylogenetic analysis

The mitochondrial DNA (mtDNA) cox2 gene was amplified using primers 210 (forward: 5′-CACAACCTTTAAAAATTTC-3′) and 211 (reverse: 5′-TTTCTAGGTATAGTTGRTTATY-3′), with the conditions described by Nadler and Hudspeth (2000). The amplified products were purified using the AccuPrep® Gel Purification Kit (Bioneer, Korea) according to the manufacturer’s instructions. The purified PCR products were directly sequenced by using the ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, USA). The obtained sequences were aligned with the published sequences in the GenBank database (NCBI) using Clustal W (Thompson et al., 1994). The phylogenetic tree was constructed by using MEGA version 5 (Tamura et al., 2011), and the genetic relationship was compared by neighbor-joining criteria. The nucleotide sequences were registered in GenBank.

### 3. Results

#### 3.1. Infection levels

The infection levels are summarized in Table 2. P was 55.4% (231/417) and MI was 7.0 (1628/231) in total. For fish samples in 2011, more than 50% of fish from each sampling location were infected with nematodes, with P being the highest (76.9%) in E-4 and the lowest (51.6%) in W-1. MI was highest (9.5) in W-1 and lowest (3.0) in E-1 (Table 2). For fish samples in 2012, the highest P (93.3%) was observed in S-1 and the lowest (18.2%) in E-3. MI was highest (10.0) in W-1 and lowest (1.1) in E-1 (Table 2).

In the sampling locations off the east coast, the total number of nematodes found and the overall P in 2011 were higher than those in 2012. In the sampling locations off the south and west coasts, hundreds of nematodes were consistently found in both 2011 and 2012 (Table 2).

Most of the nematodes were found freely in the body cavity of chub mackerel (91.7% in 2011 and 98.8% in 2012), regardless of sampling locations (Table 2). Some were attached to the serosal surfaces of the stomach and intestines or encapsulated on the surfaces of the gonads and liver.

#### 3.2. Identification of nematodes by PCR-RFLP

All of the isolated nematodes were identified by PCR-RFLP pattern analysis. The amplification of the ribosomal DNA region produced an approximately 1 kb fragment (data not shown). RFLP analysis using HinfI, Rsal, and HaeIII restriction enzymes revealed 4 different banding patterns. Digestion of the PCR product with HinfI produced 4 different banding patterns: 350–300–250 bp, 650–350–250 bp, 650–250 bp, and 700–350 bp (Fig. 2A). Digestion using Rsal produced 2 different patterns: 550–300 bp and 650–220 bp (Fig. 2B). Digestion using HaeIII produced 2 different patterns: 650–200 bp and 350–220–200–100 bp (Fig. 2C). The banding patterns found in this study corresponded to the known patterns of A. pegreffii, a hybrid genotype (A. simplex (s.s.) × A. pegreffii), and A. simplex (s.s.) (Lanes 1, 2, and 3, respectively, in Fig. 2A, B, C). One RFLP pattern different from those of genus Anisakis mentioned above was also found (Lane 4 in Fig. 2A, B, C). The most frequently identified nematode was A. pegreffii (94.3%; 1535/1628). The second most frequently identified one was Hysterothylacium sp. (28.8%; 46/1628). The hybrid genotype (A. simplex (s.s.) × A. pegreffii) and A. simplex (s.s.) were 2.5% (41/1628) and 0.4% (6/1628) of the identified nematodes, respectively (Table 3).

#### 3.3. Mitochondrial cox2 gene DNA sequencing and phylogenetic analysis

Because a large number of A. pegreffii (1535/1628) were found by PCR-RFLP in this study, randomly selected samples of A. pegreffii were sequenced targeting the mt cox2 gene. For other species, all of the samples were sequenced.

All of the sequenced samples showing the novel RFLP patterns of A. pegreffii in this study (GenBank accession number: JX091680–JX091677, JF825529–JF825530, KC633412–KC633434) had 97.8–100% similarities with the previously described sequences of A. pegreffii from Japanese chub mackerel (GenBank accession number: AB517562) and Korean common squid (T. japonicus) (GenBank accession number: JF072744) (Table 4). The sequences of the samples showing A. simplex RFLP patterns in this study (GenBank accession number: KC633435–KC633436, KC633441) had 98.6–99.7% similarities with the previously sequenced A. simplex (s.s.) of Korean chum salmon (Oncorhynchus keta) (GenBank accession number: HM489002) and Korean common squid (GenBank accession number: HQ702736) (Table 4). In phylogenetic analysis, all of them were clustered with those previously identified as A. pegreffii and A. simplex (s.s.), respectively (Fig. 3). All of the hybrid genotype (A. simplex (s.s.) × A. pegreffii) samples were found to be A. pegreffii by mt cox2 gene sequencing (data now shown). In addition, the samples...
showing patterns that did not correspond to those of novel Anisakis species (GenBank accession number: KC633437–KC633440, KC633442–KC633450) showed 97.6–100% similarities with Hysterothylacium sp. previously described in Korean pacific cod (Gadus macrocephalus; GenBank accession number: HM437221) by the sequencing of the mt cox2 gene (Table 5).

### 3.4. Geographical distribution of anisakid nematodes from chub mackerel around the Korean Peninsula

The prevalence of each anisakid nematode species in chub mackerel from each sampling location is summarized in Table 3. The most frequently found nematode during sampling periods in each sampling location was A. pegreffii, except for Hysterothylacium sp. at E-4 in 2012. In 2011, the average percentage of A. pegreffii in the identified nematodes was 94.4%, with the highest percentage being 96.4% in S-2. In 2012, the average percentage of A. pegreffii was 93.9%, with the highest percentage of 100% in E-3. The second most commonly identified nematode was the hybrid genotype (A. simplex (s.s.) × A. pegreffii) in 2011 and Hysterothyacium sp. in 2012, but the overall percentages were very low (2.8% for the hybrid genotype in 2011 and 3.5% for Hysterothyacium sp. in 2012). A. simplex (s.s.) was found only in some sampling locations and the percentage in each sampling location was low (0–12.5%).

### 4. Discussion

The chub mackerel (S. japonicus) is a pelagic fish distributed in temperate and subtropical zones throughout the world. It feeds on planktonic crustaceans, particularly euphausiids and small fish such as anchovy (Engraulis japonicus) (Yoon et al., 2008). Many of these prey items are known as intermediate/paratenic hosts of anisakid nematodes (Hays et al., 1998; Rello et al., 2009), and several studies have shown that chub mackerel in different areas are infected with diverse species of anisakid nematodes (Abattouy et al., 2011; Shukhgalter, 2004; Suzuki et al., 2010). In addition, other scombrid fish species such as S. colias, S. australasicus, and S. scombrus are known to be infected with various anisakid nematodes species (Chou et al., 2011; Costa et al., 2011; Gutierrez-Galindo et al., 2010). Given that these scombrid fish are commercially important fish species, anisakid nematode larvae can be accidentally transmitted to humans when the fish are consumed raw or undercooked. In fact, chub mackerel has been considered to be the main source of human anisakidosis in Japan (Oshima, 1987; Umehara et al., 2007). Moreover, the presence of anisakid nematodes in chub mackerel can cause an esthetic problem of consumers to reject

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Sampling locations</th>
<th>P (%)</th>
<th>MI (%)</th>
<th>% of nematodes (number of nematodes/total number of nematodes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body cavity</td>
<td>Stomach</td>
<td>Intestine</td>
<td>Liver</td>
</tr>
<tr>
<td>2011.11.13</td>
<td>E-1</td>
<td>52.7 (29/55)</td>
<td>3.0 (88/29)</td>
<td>92.1 (81/88)</td>
</tr>
<tr>
<td>10.12</td>
<td>E-4</td>
<td>76.9 (50/65)</td>
<td>7.1 (353/50)</td>
<td>95.2 (336/353)</td>
</tr>
<tr>
<td>10.25</td>
<td>S-2</td>
<td>613 (49/80)</td>
<td>51.1 (447/90)</td>
<td>87.5 (291/447)</td>
</tr>
<tr>
<td>11.17</td>
<td>W-1</td>
<td>516 (33/64)</td>
<td>9.5 (313/33)</td>
<td>93.6 (293/313)</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>610 (161/264)</td>
<td>7.5 (1201/161)</td>
<td>917 (1101/1201)</td>
</tr>
<tr>
<td>2012.10.18</td>
<td>E-1</td>
<td>25.0 (5/20)</td>
<td>1.8 (9/5)</td>
<td>100 (9/9)</td>
</tr>
<tr>
<td>10.14</td>
<td>E-2</td>
<td>23.3 (7/30)</td>
<td>1.9 (13/7)</td>
<td>84.6 (11/13)</td>
</tr>
<tr>
<td>10.24</td>
<td>E-3</td>
<td>18.2 (4/22)</td>
<td>2.3 (9/4)</td>
<td>100 (9/9)</td>
</tr>
<tr>
<td>11.30</td>
<td>E-4</td>
<td>233 (7/30)</td>
<td>1.1 (8/7)</td>
<td>75.0 (6/8)</td>
</tr>
<tr>
<td>10.14</td>
<td>S-1</td>
<td>93.3 (28/30)</td>
<td>7.1 (198/28)</td>
<td>100 (198/198)</td>
</tr>
<tr>
<td>11.15</td>
<td>W-1</td>
<td>90.5 (19/21)</td>
<td>10.0 (190/19)</td>
<td>99.5 (189/190)</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>45.8 (70/153)</td>
<td>6.1 (427/70)</td>
<td>98.8 (422/427)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>55.4 (231/417)</td>
<td>7.0 (1628/231)</td>
<td>93.6 (1523/1628)</td>
</tr>
</tbody>
</table>

* Prevalence of infection.
* Mean intensity.

**Fig. 2.** PCR-RFLP profiles of anisakid nematodes obtained by digestion of the PCR-amplified ITS region with HinfI (Fig. 2A), Rsal (Fig. 2B), and HaeIII (Fig. 2C) restriction enzymes, respectively. (L: ladder, 1: Anisakis pegreffii, 2: hybrid genotype, 3: Anisakis simplex (s.s.), 4: Hysterothyacium sp.)
the fish. Although chub mackerel is considered to be a commercially important fish species and has been suspected to be one of the main sources of human anisakidosis in Korea, no systemic survey has been conducted yet. Thus, it is important to investigate the anisakid nematode fauna of chub mackerel to assess the risk of human anisakidosis from the ingestion of chub mackerel in Korea.

Kang et al. (2008) and Lee et al. (2009) reported Anisakis spp. and A. pegreffii from chub mackerel in Korea by molecular analysis. However, their studies were conducted with a small number of fish samples bought from local inland fisheries markets, where the origins of chub mackerel are not always clear. In this study, we assessed the infection levels and diversity of anisakid nematodes in chub mackerel from Korean waters over 2 years. A. pegreffii, A. simplex (s.s.), a hybrid genotype (A. simplex (s.s.) × A. pegreffii), and Hysterothylacium sp. were identified by molecular analysis. A. pegreffii was the most abundant species (1535/1628; 94.3%) and Hysterothylacium sp. was the second most abundant species (46/1628; 2.8%).

Anisakis pegreffii Campana-Rouget and Biocca, 1955 is widely distributed in the Austral region between 30°N and 55°S, as well as in the Mediterranean Sea, and several fish and squids are known to be its intermediate/paratenic hosts (Mattucci and Nascetti, 2008 and the references therein). A. pegreffii has been reported in various fish species and cephalopods caught from the East Asian Pacific regions (Du et al., 2010; Setyobudi et al., 2013; Umehara et al., 2006). In particular, it has been reported in chub mackerel caught from the Pacific and the Mediterranean Sea (Abattouy et al., 2011; Suzuki et al., 2010; Vardić Smržić et al., 2012). These results suggest that chub mackerel is a suitable paratenic/transport host for A. pegreffii and reflect that dolphins, mainly of family Delphinidae (known suitable definitive hosts of A. pegreffii), are available in these waters.

Genus Hysterothylacium Ward & Magath, 1917 is a common nematode parasite of teleosts, especially in marine environments (Navone et al., 1998). Currently this genus includes more than 70 described species, and of them, Hysterothylacium aduncum is the most frequently recorded species worldwide (Li et al., 2013 and the references therein). While sexually mature adults are found in the digestive tracts of various marine fish species, their third and fourth larval stages can be found in various tissues of numerous marine fishes and invertebrates (Bruce et al., 1994). Scombrid fish, including chub mackerel, have been reported to harbor H. aduncum (Chou et al., 2011; Navone et al., 1998; Vardić Smržić et al., 2012). In this study, Hysterothylacium sp. was found in chub mackerel using PCR-RFLP and the direct sequencing of the mt cox2 region. However, clear identification at the species level and the taxonomic positioning of Hysterothylacium sp. were not possible in this study because morphological observations were not made. Moreover, the amount of mt cox2 gene sequence data deposited in the GenBank was not sufficient to conduct a phylogenetic analysis. Many species of Hysterothylacium have been poorly described, which makes it difficult to compare newly described species with the previously described species (Bruce et al., 1994). Recently, Shamsi et al. (2013)
described several different *Hysterothylacium* larvae by morphology and genetically characterized them by ITS sequence analysis, but could not find clear match between the sequences they found and those in GenBank. Clear identification of the *Hysterothylacium* sp. found here awaits further study.

*Anisakis simplex* (Rudolphi, 1809) (sensu stricto) is widespread in the Northern Hemisphere. At least 9 cetacean species are known to be its definitive hosts, and many fish species, including chub mackerel, and squids are known to harbor its larvae throughout its geographical range (Mattiucci and Nascetti, 2008). In particular, *A. simplex* (s.s.) is the sequences obtained in this study.

Fig. 3. Molecular phylogenetic tree showing the genetic relationships among *Anisakis* species based on mtDNAcox2 gene sequences. Analysis was performed using the MEGA5 program. The scale bar indicates distance.

| Accession no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|
| 1  | KC633437* | 100 | 97.8 | 98.1 | 99.1 | 98.6 | 98.1 | 99.3 | 99.1 | 98.4 | 99.3 | 98.8 | 99.1 | 98.1 | 83.2 | 90.3 | 80.7 | 81.2 | 81.9 |
| 2  | KC633438* | 100 | 97.8 | 98.1 | 99.1 | 99.0 | 98.4 | 99.7 | 99.5 | 98.8 | 99.3 | 99.1 | 99.5 | 98.4 | 83.4 | 90.7 | 80.5 | 81.7 | 81.9 |
| 3  | KC633439* | 100 | 99.0 | 97.6 | 98.4 | 98.3 | 98.1 | 97.9 | 98.6 | 97.8 | 97.6 | 97.9 | 98.3 | 83.6 | 90.3 | 81.0 | 81.9 | 82.7 |
| 4  | KC633440* | 100 | 97.9 | 98.8 | 98.6 | 98.4 | 98.3 | 98.6 | 98.1 | 97.9 | 98.3 | 98.3 | 83.2 | 90.3 | 80.8 | 81.5 | 82.4 |
| 5  | KC633441* | 100 | 98.8 | 98.3 | 99.5 | 99.3 | 98.6 | 99.1 | 99.0 | 99.3 | 96.3 | 83.2 | 90.8 | 80.1 | 82.0 | 82.6 |
| 6  | KC633443* | 100 | 99.1 | 99.3 | 99.1 | 99.1 | 99.0 | 98.8 | 99.5 | 98.8 | 98.3 | 83.4 | 91.4 | 80.8 | 81.5 | 82.9 |
| 7  | KC633444* | 100 | 98.8 | 98.8 | 98.6 | 98.4 | 98.3 | 98.6 | 98.3 | 83.6 | 91.0 | 80.8 | 81.0 | 83.1 |
| 8  | KC633445* | 100 | 99.8 | 99.1 | 99.7 | 99.5 | 99.8 | 98.8 | 83.4 | 90.7 | 80.5 | 81.5 | 82.2 |
| 9  | KC633446* | 100 | 99.0 | 99.5 | 99.3 | 99.7 | 98.6 | 83.4 | 90.7 | 80.5 | 81.7 | 82.2 |
| 10 | KC633447* | 100 | 98.8 | 98.8 | 98.6 | 99 | 99.7 | 83.9 | 90.8 | 80.5 | 81.7 | 82.7 |
| 11 | KC633448* | 100 | 99.1 | 99.5 | 98.4 | 98.3 | 83.4 | 90.3 | 80.5 | 81.2 | 82.2 |
| 12 | KC633449* | 100 | 99.3 | 98.3 | 98.4 | 90.2 | 80.1 | 81.5 | 82.0 |
| 13 | KC633450* | 100 | 98.6 | 83.2 | 90.8 | 80.3 | 81.7 | 82.4 |
| 14 | HM437221 | 100 | 84.1 | 91.2 | 80.5 | 81.9 | 82.7 |
| 15 | JF302065 | 100 | 83.4 | 83.2 | 80.5 | 83.1 |
| 16 | JQ934891 | 100 | 80.8 | 82.4 | 83.4 |
| 17 | AF179914 | 100 | 77.5 | 80.0 |
| 18 | AF179915 | 100 | 80.7 |
| 19 | AF179916 | 100 |

1–4 Hysterothylacium sp. (E-1), 5–6 Hysterothylacium sp. (E-2), 7–9 Hysterothylacium sp. (E-4), 10–11 Hysterothylacium sp. (S-1), 12–13 Hysterothylacium sp. (W-1), 14 Hysterothylacium sp. (isolated in pacific cod from Korea), 15 Hysterothylacium deardorffoverstreetorum, 16 Hysterothylacium aduncum, 17 Hysterothylacium fortalezae, 18 Hysterothylacium pelagicum, and 19 Hysterothylacium reliquens.
known to be commonly found in chub mackerel of the Pacific stock and is epidemiologically important for human anisakidosis in Japan (Suzuki et al., 2010). The hybrid genotype (A. simplx (s.s.) × A. pegreffii) has been also reported from several distant geographical regions and seems to occur worldwide where A. pegreffii and A. simplx (s.s.) occur in sympatry (Abollo et al., 2003; Du et al., 2010). It is not clear if this hybrid genotype can mate and produce offspring.

The geographical origins of fish are important for studying the epidemiology of anisakidosis. Fish can show different parasitic assemblages depending on their geographical origins. For example, chub mackerel belonging to the Pacific stock harbored A. simplx (s.s.), whereas chub mackerel belonging to the Tsushima stock harbored A. pegreffii (Suzuki et al., 2010). This is thought to reflect the different availability of invertebrates that are intermediate/paratenic hosts of anisakid nematodes as food items to chub mackerel. In addition, different geographical distributions of final cetacean hosts of each anisakid nematode species are believed to be involved. These different anisakid nematode assemblages of fish hosts are assumed to consequently affect the probability of humans contracting anisakid nematodes, depending on the geographical origins of fish.

In this study, differences in the prevalence of infection and the assemblages of anisakid nematodes in chub mackerel were observed depending on sampling years. In particular, the prevalence of infections in chub mackerel caught from the eastern area of Korea fluctuated, i.e., it was higher in 2011 than in 2012. This could possibly be due to the changes in the availability of invertebrates in the sampling areas, as mentioned above. Seong et al. (2010) observed that the sea water temperature at 100 m depth has been decreasing in the sea east of the Korean Peninsula, although the overall sea water temperature around the Korean Peninsula has been increasing. Climate changes can influence the occurrence and abundance of anisakid nematodes, directly influencing their free-living larval stages and indirectly influencing their invertebrate and vertebrate hosts (Rokicki, 2009). Annual fluctuation in the prevalence of anisakid nematodes from the sea east of the Korean Peninsula may be related to the changes in sea water temperature mentioned above, but more extensive studies over a longer period will be necessary because many biological, ecological, climatic, and trophic factors affect the population dynamics of the intermediate and definitive hosts of anisakid nematodes.

Chub mackerel in Korean waters are known to consist of 2 different stocks: the Tsushima Current stock and the East China Sea stock, although the latter is assumed to be the same as the Tsushima stock: the Tsushima Current stock and the East China Sea stock, locations around the Korean Peninsula. Of them, diet and trophic factors affect the population dynamics of the intermediate and will be necessary because many biological, ecological, climatic, and geographical distributions of the anisakid nematodes in chub mackerel were observed from these locations were similar to those of fish samples from the Tsushima Current stock previously investigated in Japan (Suzuki et al., 2010), i.e., A. pegreffii was the most abundant species in chub mackerel. Fish samples caught from west of the Korean Peninsula (W-1), geographically distant from Tsushima Island, also showed similar anisakid nematode assemblages to those of the Tsushima Current stock mentioned above. In addition, the anisakid nematode species assemblages differed between Korea and Japan. Chub mackerel samples around the Korean Peninsula harbored diverse anisakid nematode species, while Japanese chub mackerel samples harbored mainly A. pegreffii in the Tsushima stock and A. simplx (s.s.) in the Pacific stock. There can be several possible explanations for this phenomenon as mentioned above, and this is thought to reflect the different migration routes of the Korean chub mackerel population and the Japanese chub mackerel population.

Although chub mackerel has been suspected to be one of the main causes of anisakidosis (Audicana and Kennedy, 2008), it still remains unclear how much it contributes to human anisakidosis in Korea because the predominant nematode found in chub mackerel was A. pegreffii, and A. simplx (s.s.) was rarely found in this study. In addition, most of the nematodes were found in the body cavity and only few in the viscera. Recently, it was suggested that A. pegreffii is less invasive than A. simplx (s.s.) by in vitro and in vivo studies (Arizono et al., 2012; Romero et al., 2013), and our results also indicate that A. pegreffii may not be sufficiently invasive to penetrate muscles in chub mackerel, as suggested by several authors (Arizono et al., 2012; Suzuki et al., 2010). Considering that chub mackerel is mostly consumed as a cooked dish and only the sliced muscle is served when eaten raw, the risk of anisakidosis to humans from eating chub mackerel may be lower than previously thought in Korea. If the anisakid nematodes isolated from human patients are clearly identified, the epidemiological role of chub mackerel in Korea will be clarified because the molecular identification of anisakid nematodes obtained from human anisakidosis in Korea has not yet been conducted, and most of them have been conventionally diagnosed as A. simplx or Anisakis type I larvae by endoscopy, histopathology, or morphological observation of the surgically removed worms (Im et al., 1995; Kim et al., 2006b, 2013).

Acknowledgment

This research was a part of the projects ‘Long-term change in structure and function in marine ecosystems of Korea’ and ‘East Asian Seas Time Series-I (EAST-I)’ funded by the Ministry of Oceans and Fisheries, Korea.

References


