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Veterinary Parasitology

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Short communication

Anisakidae infection in fish of the Aegean Sea

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ARTICLE INFO

Article history:

Received 2 May 2011

Received in revised form 31 August 2011

Accepted 6 September 2011

Keywords:

Anisakis simplex

Anisakis pegreffii

Hysterothylacium sp.

fish

Aegean Sea

Greece

ABSTRACT

Nematode worms of the family Anisakidae are the causative agents of infections in humans when fish is consumed raw and of serious allergies up to the death, when fish is consumed raw or cooked by previously sensitized people. From April until November 2009, 462 fish belonging to 26 species, fished in three areas of the Aegean Sea were tested for Anisakidae larvae. Anisakidae larvae were detected in 87 (18.83%) fish of 13 species. These larvae were identified by morphology as the third-stage larvae of the genera *Hysterothylacium* sp. or *Anisakis*. Larvae of the genus *Anisakis* were identified by PCR-RFLP as belonging to *A. simplex* s.str., *A. pegreffii*, or as hybrids between *A. simplex* s.str. and *A. pegreffii*.

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1. Introduction

Nematodes of the family Anisakidae have a global distribution among a wide variety of marine fish species that serve as intermediate or paratenic hosts (Køie et al., 1995). Marine fish become infected ingesting crustaceans or other fish infected by the third-stage larvae (L₃). L₃ develop into the adult stage in marine mammals, such as whales and dolphins (definitive hosts), when they ingest infected fish (Sakanari and Mckerrow, 1989).

Humans acquire the infection by the accidental ingestion of larvae present in raw or undercooked fish parasitized by larvae of the genera *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* (Hochberg and Hamer, 2010). The infection can cause digestive disorders and/or allergies known as Anisakiasis (Daschner et al., 2000; Audicana and Kennedy, 2008).

About 20,000 cases of Anisakiasis were reported worldwide, but over 90% of them are from Japan and 10% from the European Union, United States of America, Canada, New Zealand, Chile and Egypt (Chai et al., 2005; Abe et al., 2006; Umehara et al., 2007).

Although there are various reports on the circulation of these zoonotic parasites in the Mediterranean Sea (Manfredi et al., 2000; Valero et al., 2000; Farjallah et al., 2008), there are no reports on human Anisakiasis in Greece and on the prevalence of Anisakidae worms in commercial fish species from the Aegean Sea. The aims of this study were to investigate the prevalence of Anisakidae larvae in fish fished from the Aegean Sea and marketed in Greece and to identify the larvae at the species level.

2. Materials and methods

2.1. Study area

Fish were collected from four fish markets of Greece from April until November, 2009. The fish markets were

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selected for the fish origin in order to cover the North, Central and South Aegean Sea.

2.2. Hosts and parasites

Out of the 462 sampled fish belonging to 26 species, 300 were from the Northern, 108 from the Central and 54 from the Southern Aegean Sea. Fish were randomly sampled during State Veterinary Authorities inspection in order to ensure that only fish approved for human consumption will be included in this study. Fish were stored in individual plastic bags in ice boxes up to the laboratory where they were frozen at -30°C until dissection.

After thawing, fish were identified at the species level, measured and weighed to the nearest 0.1 cm and 0.1 g, respectively; they were dissected to separate the viscera from the muscle tissues and then both tissues and viscera were separately digested by a pepsin–HCl solution. Nematode larvae were collected from the digestion liquid, counted and washed with 0.9% NaCl solution, and preserved in 70% ethanol for further identification. After clarification in lactophenol, larvae were identified at the genus level by a light microscope, according to the morphological criteria proposed by Berland (1961), Peter and Maillard (1988), and Peter and Cabaret (1995).

2.3. Genomic DNA extraction and PCR-RFLP analysis

The genomic DNA (gDNA) was extracted from the worm using the “Tissue and Hair extraction kit” (Promega, Italy) combined with the “DNA IQ system kit” (Promega) and eluted in a final volume of 100 μl . The rDNA region of approximately 1Kb, comprising the ITS-1 region, 5.8S gene, ITS-2 region plus approximately 70 nucleotides of the 28S gene, was PCR amplified using the oligonucleotides NC5 (forward; 5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse; 5'-TTAGTTTCTTTTCCTCCGCT-3') from 2 μl of the extracted gDNA and gDNA of *A. pegreffii* was used as control. The PCR reaction was performed according to the protocol described in Zhu et al. (1998), using the Go-Taq master mix DNA polymerase (Promega) in a T-personal thermocycler (Whatman-Biometra, Germany). Aliquots (5–10 μl) of individual PCR products were digested with 10 units of either the restriction endonuclease HinfI or HhaI in a final volume of 20 μl at 37°C for 16 h according to D'Amelio et al. (2000) and Abollo et al. (2003). The theoretical restriction fragments profile for *Hysterothylacium* spp. was obtained by alignment and *in silico* digestion of the sequences of *H. auctum* (GenBank: AF115571.1), *Hysterothylacium* sp. JYW-2010 (GenBank: HM545895.1) and *H. bidentatum* (GenBank: AY603539.1) encompassing, partially, the internal transcribed spacer 1, the complete 5.8S ribosomal RNA gene and, partially, the internal transcribed spacer 2. ClustalW2 application (<http://www.ebi.ac.uk/Tools/msa/clustalw2>) was used for sequence alignment and Webcutter 2.0 application (<http://www.firstmarket.com/cutter/cut2.html>) for *in silico* digestion with HhaI and HinfI enzymes. Digestion products were separated by the Qiaxcel capillary electrophoresis apparatus (Qiagen GmbH, Hilden, Germany) and the runs were visualized and analyzed using the

Table 1
Fish species from the Aegean sea tested for Anisakidae larvae and their level of infection.

Fish species	Mean length (range)	Mean weight (range)	Infected/tested (%)	Mean intensity of infection	Mean abundance of infection	Mean number of larvae (range)	Number of collected larvae
<i>Merluccius merluccius</i>	26.8 (21–33)	136.5 (61–252)	24/36(66.6)	8.9	5.9	5.97 (1–99)	215
<i>Micromesistius poulassou</i>	24.7 (22–27.5)	105.2 (74–138)	4/4	17	17	17(3–34)	68
<i>Scomber japonicus colias</i>	28.4 (24–36)	219.5 (108–442)	24/32(75)	13.8	10.3	10.3 (1–90)	331
<i>Scomber scombrus</i>	31.2 (27–33)	252.7 (178.5–334)	5/5	14	14	14(2–55)	70
<i>Trachurus trachurus</i>	20.4 (16.5–31)	83.9 (37–235)	16/40(40)	18	7.17	7.2 (1–84)	287
<i>Boops boops</i>	17.2 (12–22)	59.6 (22–112.5)	2/46(4.3)	4	0.2	0.17 (2–6)	8
<i>Diplodus anularis</i>	14.9 (13.9–16)	51.03 (44–57.5)	2/5	1	0.4	0.4 (1)	2
<i>Engraulis encrasicolus</i>	12.4 (10–15)	13.2 (6–26)	3/77(3.9)	1.3	0.05	0.05 (1–2)	4
<i>Mullus barbatus</i>	14.05 (9.5–19)	34.3 (6.5–73)	1/18(5.5)	1	0.05	0.05 (1)	1
<i>Pagellus erithrinus</i>	14.6 (11–21.7)	50.5 (31–140.5)	2/17(11.8)	1	0.1	0.12 (1)	2
<i>Sardina pilchardus</i>	13.4 (12–17)	20.5 (15–46)	2/36(5.5)	1	0.05	0.05 (1)	2
<i>Sardinella aurita</i>	18.9 (15–22)	58.03 (41.5–95)	1/22(4.5)	1	0.04	0.04 (1)	1
<i>Spicara smaris</i>	11.8 (10–13.5)	14.4 (10–22.5)	1/26(3.8)	1	0.04	0.04 (1)	1

Table 2L₃ larvae of the Anisakidae family detected in fish of the Aegean Sea.

Host	N. of fish with Anisakidae larvae	<i>A. pegreffi</i>	<i>A. simplex</i> s.s.	<i>A. pegreffi</i> / <i>A. simplex</i>	<i>Hysterothylacium</i> sp.
North Aegean					
<i>Merluccius merluccius</i>	18	14	3		1
<i>Engraulis encrasicolus</i>	2	2			
<i>Scomber japonicus colias</i>	17	12	3	2	
<i>Micromesistius poutassou</i>	4	1	1	2	
<i>Trachurus trachurus</i>	3	2			1
<i>Scomber scombrus</i>	5	1	2	1	1
<i>Mullus barbatus</i>	1		1		
<i>Diplodus anulus</i>	2	2			
South Aegean					
<i>Pagellus erithrinus</i>	1			1	
<i>Boops boops</i>	1				1
<i>Spicara smaris</i>	1	1			
<i>Sardina pilchardus</i>	1	1			
Central Aegean					
<i>Scomber japonicus colias</i>	2	2			
<i>Trachurus trachurus</i>	10	8		2	
Total	68	46 (67.6%)	10 (14.7%)	8 (11.7%)	4 (5.8%)

BioCalculator software. The single runs were aligned using as reference the electrophoretic position of the 15 bp–1 Kb alignment markers.

3. Results and discussion

Out of the 462 examined fish belonging to 26 species, 87 (18.83%) of 13 species harbored Anisakidae larvae, with a mean intensity of 11.4 and a mean abundance of 2.15. For each parasitized fish species the infection indexes as well as length and body weight measures are shown in Table 1.

A total of 992 Anisakidae larvae from both viscera and muscles, were collected and identified by morphology as L₃ larvae of *Anisakis* type I or *Hysterothylacium* sp. Atlantic mackerel (*Scomber scombrus*), blue whiting (*Micromesistius poutassou*), chub mackerel (*Scomber japonicus colias*), European hake (*Merluccius merluccius*), annular seabream (*Diplodus annularis*) and Atlantic horse mackerel (*Trachurus trachurus*), were the most highly infected species. The higher parasite burden was detected in *S. japonicus colias* while the higher mean intensity was found in *T. trachurus* (Table 1). No L₃ was detected in: 2 false scads (*Caranx rhonchus*); 3 crevalle jacks (*Caranx hippos*); 2 bullet tunas (*Auxis rochei*); 3 garfish (*Belone belone*); 13 goldstriemes (*Boops salpa*); 5 sand steenbrases (*Lithognathus mormurus*); 19 black gobies (*Gobius niger*); 16 deep-water rose shrimps (*Parapenaeus longirostris*); 8 mullets (*Mugilidae*); 7 saddle breams (*Oblada melanura*); 7 black seabreams (*Scatharus graecus* syn *Spondylusoma cantharus*); 9 European barracudas (*Sphyraena sphyraena*) and 4 Mediterranean horse mackerels (*Trachurus mediterraneus*).

Larvae collected from 87 fish samples (up to three larvae for each sample) were subjected to species identification by molecular analysis. Only larvae from 69 fish samples were PCR positive and could be further identified at the species level (Fig. 1, Table 2). All the three identified larvae collected from single fish showed the same species profile. In one case, the PCR-RLFP profile was not assigned. The majority of the fish (66, 95%) were infected with larvae belonging to the *A. simplex* complex (*A. simplex* s.l.); of them, 46 fish

(71%) were infected with *A. pegreffi*. Fish harboring *A. pegreffi* originated from the whole Aegean Sea. L₃ larvae (15%) identified as *A. simplex* s.s., were detected in 10 fish of the North Aegean sea. Nine larvae (14%) identified as an *A. pegreffi*/*A. simplex* hybrid, were detected in *M. poutassou* and *S. japonicus colias* from the North Aegean sea, in 1 common pandora (*Pagellus erithrinus*) from the South Aegean sea and in a *T. trachurus* from the Central Aegean sea. *Hysterothylacium* sp. larvae were identified in 1 *B. boops*, 1 *M. merluccius*, 1 *S. scombrus* and 1 *T. trachurus*, from the Central and the South Aegean sea.

The results show that Anisakidae larvae commonly parasitize a great variety of marketed fish in Greece similarly to data collected in a number of surveys carried out in most of the Mediterranean Sea along the European and African coasts (Papoutsoglou, 1975; Kosuth et al., 2001; Abollo et al., 2003; Costa et al., 2004; Farjallah et al., 2008; Rello et al., 2009).

In agreement to previously published works, *M. merluccius*, *M. poutassou*, *Scomber* spp. and *Trachurus* spp. are confirmed as the most commonly infected fish species (Manfredi et al., 2000; Valero et al., 2000; Fernandez et al., 2005; Farjallah et al., 2008; Gutiérrez-Galindo et al., 2010).

In the few studies carried out on fish collected from European markets, the species identification of Anisakidae larvae based only on the L₃ morphology has shown the *A. simplex* complex as the most prevalent Anisakidae species (Chord-Auger et al., 1995; Adroher et al., 1996). For example in Belgian fish markets, 13% of fish harbored *A. simplex* L₃ which is similar to our prevalence of 18% detected in fish from Greek markets (Piccolo et al., 1999). However, in the Belgian survey, the pollock (*Pollachius pollachius*) was the most frequently infected fish (Piccolo et al., 1999), reflecting the different origin of the marketed fish between the North sea and Northeast Atlantic ocean, and the Mediterranean sea.

In agreement to previously published works carried out in the Mediterranean sea (Mattiucci and Nascetti, 2008), *A. pegreffi* was the most prevalent species detected in the Aegean Sea. Intriguingly, the *A. pegreffi*/*A. simplex*

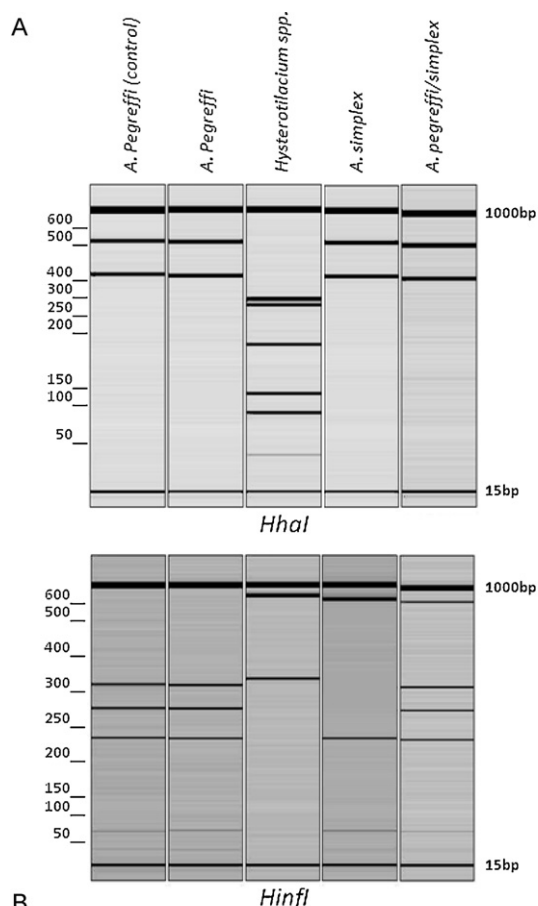


Fig. 1. Genotyping of worms. (A) Graphic output of the run and analysis with BioCalculator software of the capillary electrophoretic separation of PCR products digested either with the restriction enzyme *HhaI* or *HinfI*. Molecular markers are on the left (base pair, bp) and alignment markers (1000 and 15 bp) are on the right. (B) Expected PCR/RFLP fragments size (base pair, bp) for each Anisakidae species with the reported restriction enzyme.

recombinant genotype was prevalent in mackerels as also reported along the Iberian Peninsula coasts (Abollo et al., 2003). This recombinant genotype has been described as the product of inter-specific hybridization occurring in areas of sympatry of the two sibling species, such as the Spanish coasts (Abollo et al., 2003; Martín-Sánchez et al., 2005), the Pacific coasts of Japan (Umehara et al., 2006) and, recently, the Yellow Sea of China (Du et al., 2010). The percentage rate of *A. pegreffii* versus *A. simplex* s.s. of our study is in agreement with that observed along the Spanish Mediterranean coasts (Martín-Sánchez et al., 2005).

In Greece, the lack of clinically reported human cases as well as of documented allergies versus Anisakidae allergens, does not necessary implies the absence of contacts between the human beings and these nematodes. However, the diagnose failure, asymptomatic infections, and/or

spontaneous healing, cannot be excluded (Deardorff et al., 1986). A survey on fish consumption within 10 European countries reported a high fish intake in the Spanish and Greek population (Welch et al., 2002). In Greece, the native feeding behavior of well-cooked meals (Rohrmann et al., 2002) can prevent infection but not allergies. However, changes of feeding habits as consequence of the globalization combined with the high prevalence of Anisakidae worms in marketed fish from the Aegean Sea, could represent a risk to acquire food-borne parasitic infection/allergies also in Greece.

References

- Abe, N., Tominaga, K., Kimata, I., 2006. Usefulness of PCR-restriction fragment length polymorphism analysis of the internal transcribed spacer region of rDNA for identification of *Anisakis simplex* complex. *Jpn. J. Infect. Dis.* 59, 60–62.
- Abollo, E., Paggi, L., Pascual, S., D'Amelio, S., 2003. Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. *Infect. Genet. Evol.* 3, 175–181.
- Adroher, F.J., Valero, A., Ruiz-Valero, J., Iglesias, L., 1996. Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada, Spain. *Parasitol. Res.* 82, 319–322.
- Audicana, M.T., Kennedy, M.W., 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clin. Microbiol. Rev.* 21, 360–379.
- Berland, B., 1961. Nematodes from some Norwegian marine fishes. *Sarsia* 2, 1–50.
- Chai, J.Y., Murrel, K.D., Lymbery, A.J., 2005. Fish-borne parasitic zoonoses: status and issues. *Int. J. Parasitol.* 35, 1233–1254.
- Chord-Auger, S., Miegerville, M., Le Pape, P., 1995. Anisakiasis in the Nantes area: from fishmongers' stalls to medical offices. *Parasite* 2, 395–400 [in French].
- Costa, G., Madeira, A., Pontes, T., D'Amelio, S., 2004. Anisakid nematodes of the blackspot seabream, *Pagellus bogaraveo*, from Madeiran waters, Portugal. *Acta Parasitol.* 49, 156–161.
- D'Amelio, S., Mathiopoulos, K.D., Santos, C.P., Pugachev, O.N., Webb, S.C., Picanço, M., Paggi, L., 2000. Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase-chain-reaction-based restriction fragment length polymorphism. *Int. J. Parasitol.* 30, 223–226.
- Daschner, A., Alonso Gomez, A., Cabanas, R., Suarez de Parga, J.M., Lopez-Serrano, M.C., 2000. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease—clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. *J. Allergy Clin. Immunol.* 105, 176–181.
- Deardorff, T.L., Fukumura, T., Raybourne, R.B., 1986. Invasive anisakiasis. A case report from Hawaii. *Gastroenterology* 90, 1047–1050.
- Du, C., Zhang, L., Shi, M., Ming, Z., Hu, M., Gasser, R.B., 2010. Elucidating the identity of *Anisakis* larvae from a broad range of marine fishes from the Yellow Sea, China, using a combined electrophoretic-sequencing approach. *Electrophoresis* 31, 654–658.
- Farjallah, S., Slimane, B.B., Busi, M., Paggi, L., Amor, N., Blel, H., Said, K., D'Amelio, S., 2008. Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. *Parasitol. Res.* 102, 371–379.
- Fernandez, M., Aznar, F.J., Montero, F.E., Raga, J.A., 2005. Endoparasites of the blue whiting, *Micromesistius poutassou* from north-west Spain. *J. Helminthol.* 79, 15–21.
- Gutiérrez-Galindo, J.F., Osanz-Mur, A.C., Mora-Ventura, M.T., 2010. Occurrence and infection dynamics of anisakid larvae in *Scomber scombrus*, *Trachurus trachurus*, *Sardina pilchardus*, and *Engraulis encrasicolus* from Tarragona (NE Spain). *Food Control* 21, 1550–1555.
- Hochberg, N.S., Hamer, D.H., 2010. Anisakidosis: perils of the deep. *Clin. Infect. Dis.* 51, 806–812.
- Køie, M., Berland, B., Burt, M.D.B., 1995. Development to third-stage larva occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Can. J. Fish. Aquat. Sci.* 52, 134–139.
- Manfredi, M.T., Crosa, G., Galli, P., Ganduglia, S., 2000. Distribution of *Anisakis simplex* in fish caught in the Ligurian Sea. *Parasitol. Res.* 86, 551–553.

- Martín-Sánchez, J., Artacho-Reinoso, M.E., Díaz-Gavilán, M., Valero-López, A., 2005. Structure of *Anisakis simplex* s.l. populations in a region sympatric for *A. pegreffii* and *A. simplex* s.s. Absence of reproductive isolation between both species. *Mol. Biochem. Parasitol.* 141, 155–162.
- Mattiucci, S., Nascetti, G., 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary processes. *Adv. Parasitol.* 66, 47–148.
- Papoutsoglou, S., 1975. Study on the Metazoan Parasites of Fish in the Saronikos Gulf. Doctoral Thesis, Patras University Greece.
- Peter, A.J., Cabaret, J., 1995. Ascaridoid nematodes of teleostean fishes from the eastern North Atlantic and seas of the north of Europe. *Parasite* 2, 217–230.
- Peter, A.J., Maillard C., 1988. Larves d'ascarides parasites de poissons en Méditerranée occidentale. *Bulletin de Museum National d'Histoire Naturelle*, Paris, S4 10 (sect. A), 347–369.
- Piccolo, G., Manfredi, M.T., Hoste, L., Vercruysse, J., 1999. Anisakidae larval infection in fish fillets sold in Belgium. *Vet. Q.* 21, 66–67.
- Rello, F.J., Adroher, F.J., Benitez, R., Valero, A., 2009. The fishing area as a possible indicator of the infection by anisakids in anchovies (*Engraulis encrasicolus*) from Southwestern Europe. *Int. J. Food Microbiol.* 129, 277–281.
- Rohrmann, S., Linseisen, J., Becker, N., Norat, T., Sinha, R., Skeie, G., Lund, E., Martinez, C., Barricarte, A., Mattisson, I., Berglund, G., Welch, A., Davey, G., Overvad, K., Tjønneland, A., Clavel-Chapelon, F., Kesse, E., Lotze, G., Klipstein-Grobusch, K., Vasilopoulou, E., Polychronopoulos, E., Pala, V., Celentano, E., Bueno-de-Mesquita, H.B., Peeters, P.H.M., Riboli, E., Slimani, N., 2002. Cooking of meat and fish in Europe—results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur. J. Clin. Nutr.* 56, 1216–1230.
- Sakanari, J.A., Mckerrow, J.H., 1989. Anisakiasis. *Clin. Microbiol. Rev.* 2, 278–284.
- Kosuth, P., Letkova, V., Sotiraki, S., 2001. Endoparasitic Helminths of the marine market fish in Thessaloniki region. In: 18th International Conference of the World Association for the Advancement of Veterinary Parasitology 2001 (WAAVP 01), Stresa, Italy, Abstract Book, p. 46 D4p.
- Umehara, A., Kawakami, Y., Araki, J., Uchida, A., 2007. Molecular identification of the etiological agent of the human anisakiasis in Japan. *Parasitol. Int.* 56, 211–215.
- Umehara, A., Kawakami, Y., Matsui, T., Araki, J., Uchida, A., 2006. Molecular identification of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) from fish and cetacean in Japanese waters. *Parasitol. Int.* 55, 267–271.
- Valero, A., Martín-Sánchez, J., Reyes-Muelas, E., Adroher, F.J., 2000. Larval anisakids parasitizing the blue whiting, *Micromesistius poutassou*, from Motril Bay in the Mediterranean region of southern Spain. *J. Helminthol.* 74, 361–364.
- Welch, A.A., Lund, E., Amiano, P., Dorronsoro, M., Brustad, M., Kumle, M., Rodriguez, M., Lasheras, C., Janzon, L., Jansson, J., Luben, R., Spencer, E.A., Overvad, K., Tjønneland, A., Clavel-Chapelon, F., Linseisen, J., Klipstein-Grobusch, K., Benetou, V., Zavitsanos, X., Tumino, R., Galasso, R., Bueno-De-Mesquita, H.B., Ocké, M.C., Charrondière, U.R., Slimani, N., 2002. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr.* 5, 1273–1285.
- Zhu, X., Gasser, R.B., Podolska, M., Chilton, N.B., 1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *Int. J. Parasitol.* 28, 1911–1921.