



## Prevalence and mean intensity of *Anisakis simplex* (*sensu stricto*) in European sea bass (*Dicentrarchus labrax*) from Northeast Atlantic Ocean

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### ABSTRACT

Viscera and muscle of a total of 40 wild 1–2 kg European sea bass (*Dicentrarchus labrax*) from Northeast Atlantic (FAO area 27) were examined for Anisakidae larvae detection by digestion method. Extracted parasites were counted and mean intensity was calculated. Parasites were identified by genetic/molecular markers (allozymes and sequences analysis of the mtDNA *cox2* gene) as belonging to the species *Anisakis simplex* (*sensu stricto*). In viscera, the main localisations of the larvae were under the gastric serosa, where several parasites alive and dead were found, and intestinal serosa. The visceral prevalence was 0.950 and the mean intensity was 96.39. The main localisation of *A. simplex* (s.s.) in edible parts was in belly muscles, with a prevalence of 0.425 and a mean intensity of 1.9. This is the first record on the prevalence and mean intensity of *A. simplex* (s.s.) in European sea bass muscle. This finding has an important consequence on epidemiology of anisakiasis and public health risk assessment.

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### 1. Introduction

Anisakidosis is the human infection with third larval stage of nematodes belonging to the families Anisakidae or Raphidascaridae. *Anisakis* and *Pseudoterranova* are the two genera most associated with anisakidosis. Marine mammals and piscivorous birds are definitive hosts, whilst aquatic invertebrates and fish are intermediate or paratenic hosts. In intermediate/paratenic fish, the larvae penetrate the intestine and invade the body cavity or the muscles, where they become encapsulated as the third stage. The systematics of the species of *Anisakis* has been clarified in recent decades by the application of molecular methodologies; the actual taxonomy of the species so far recognised in this genus has been recently reviewed (Mattiucci and Nascetti, 2008). Genetic-molecular methods are particularly useful in this regard since larval stages of *Anisakis* are not recognisable by morphological characters at species level. Although humans are accidental host, ingested larvae may nevertheless attempt to penetrate the gastrointestinal wall and could cause invasive and/or allergic syndromes with different intensity (Audicana and Kennedy, 2008). People can incur in anisakiasis when consuming raw or lightly cooked seafood products. The country with the highest number of anisakiasis cases is Japan, where sushi and sashimi are traditional preparations. Other dangerous traditional preparations are: Italian alici marinate (mari-

nated anchovies) (Moschella et al. 2004), Scandinavian gravlax (raw cured salmon), Spanish bouquerones en vinagre (pickled anchovies), Hawaiian lomi-lomi (raw salmon), Filipino kinilaw (chopped, marinated fish), Latin American ceviche (raw fish seasoned with lemon juice) and Dutch smoked herrings (Chai et al., 2005). In Italy 29 identified cases of human anisakiasis are reported over the period 1996–2010; in all the cases the patient referred the consume of raw or marinated fish; anisakiasis is misdiagnosed and underestimated, in fact the diagnosis is often obtained after surgery (Biondi et al., 2008; Fumarola et al., 2009; Maggi et al., 2000; Montalto et al., 2005; Moschella et al. 2004; Pampiglione et al., 2002; Pellegrini et al., 2005; Rea et al., 2008; Testini et al., 2003; Ugenti et al., 2007; Zullo et al., 2010). In wild European sea bass (*Dicentrarchus labrax*) from Northeast Atlantic a high prevalence of Anisakidae larvae in viscera has been demonstrated (Bernardi, 2009). The increasing use of this fish species as raw product for sushi preparations in Japanese restaurants in Italy (Legnani, 2010) has suggested to extend the epidemiological survey on the presence of zoonotic anisakid larvae also to the muscle tissue.

### 2. Materials and methods

#### 2.1. Samples collection and preparation

Forty fresh European sea bass were obtained during 2010 (January–July) from Italian market. All specimens were captured in Northeast Atlantic, FAO area 27. In laboratory, the fishes were recorded and

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weighted before and after the gutting. The viscera were removed and stored in a numbered 1000 ml beaker at 4 °C. The eviscerated fish was carefully skinned and filleted obtaining 2 dorsal fillets and 2 ventral fillets with belly flaps.

## 2.2. Visual examination of muscle fillets

The four fillets obtained were carefully examined by naked eye under a magnifier desk lamp 3×. Afterwards each fillet was sliced into thin strips (about 10 mm); all the strips were examined against fluorescent light to reveal nematodes. The larvae detected were carefully isolated from muscles and frozen at –70 °C in vials for their genetic molecular identification. The inspected fillets were reserved for digestion.

## 2.3. Digestion method

Viscera and muscle were digested separately. In 1500 ml beaker, 15 g of pepsin powder was dissolved in physiologic saline solution (NaCl 0.85%). The obtained solution was adjusted to pH 2 with 6 N HCl. The beaker was placed on a magnetic stirrer with heating at 37 ± 1 °C. After adding the sample, the beaker content was stirred mechanically since the complete digestion of fish tissues. After digestion, the content was filtered through a 1 mm sieve into pan. The sieve was washed with saline solution and the residue was transferred to a Petri dish with saline solution for the count. A representative pool of larvae recovered from viscera was frozen at –70 °C for molecular identification, whilst some larvae were subjected to morphological observation.

## 2.4. Morphological identification

The larvae from viscera not analysed by molecular biology, were fixed in 70% ethanol and cleared with glycerine for the identification at genus level by light microscope according to Berland (1961).

## 2.5. Species identification

Because the larval identity is not possible based on morphologic data, the genetic molecular identification has been here used in order to identify most of the *Anisakis* larvae recovered in the examined fish. The specific identification of the larval specimens of *Anisakis* spp. recovered was performed by standard horizontal starch gel electrophoresis at those enzyme loci given as diagnostic between the *Anisakis* spp. These are: adenylate kinase (*Adk-2*, EC 2.7.4.3), leucine-aminopeptidase (*Lap-2*, EC 3.4.11.1), leucine-leucine peptidase (*Pep B*, EC 3.4.11), leucine-alanine peptidase (*PepC-1*, *PepC-2*, EC 3.4.11), mannose phosphate isomerase (*Mpi*, EC 5.3.1.8) and phosphoglucosyltransferase (*Pgm-2*, EC 5.4.2.2). The staining procedures are those reported, in detail, in Mattiucci et al. (1997). Allozymes at the diagnostic loci as given in Mattiucci and Nascetti (2006) and implemented in Mattiucci et al. (2009), were used to identify to the species level the larval *Anisakis* here collected.

Some of the *Anisakis* larvae analysed by allozymes were also sequenced, by PCR, analysing a mtDNA fragment of 629 bp of the cytochrome oxidase 2 (*cox2*). DNA was extracted using CTAB method described in Mattiucci et al. (2009); the PCR was carried out using primers 211F and 210R (Valentini et al., 2006). The sequences obtained were compared to those already observed for the same gene for all the *Anisakis* spp. and previously deposited in GenBank (Valentini et al., 2006) with their following accession numbers: *A. simplex* (s.s.) [DQ116426], *A. pegreffii* [DQ116428], *A. simplex C* [DQ116429], *A. typica* [DQ116427],

**Table 1**

Forty European sea bass from FAO area 27, sex, weight (kg), number of *A. simplex* (s.s.) in visceral body cavity and in muscle, and muscle localisation.

| Sample |                   |     | Number of larvae |         | Muscle localisation |
|--------|-------------------|-----|------------------|---------|---------------------|
|        | European sea bass | Sex | Weight           | Viscera | Muscle              |
| 1      |                   | F   | 1.60             | 47      | 0                   |
| 2      |                   | F   | 1.97             | 6       | 0                   |
| 3      |                   | F   | 1.39             | 66      | 0                   |
| 4      |                   | F   | 1.12             | 0       | 0                   |
| 5      |                   | M   | 1.90             | 30      | 0                   |
| 6      |                   | M   | 1.96             | 17      | 3                   |
| 7      |                   | M   | 1.68             | 0       | 1                   |
| 8      |                   | M   | 1.19             | 6       | 0                   |
| 9      |                   | M   | 1.17             | 35      | 0                   |
| 10     |                   | F   | 1.53             | 9       | 0                   |
| 11     |                   | F   | 1.21             | 119     | 2                   |
| 12     |                   | M   | 1.20             | 44      | 0                   |
| 13     |                   | F   | 1.20             | 60      | 0                   |
| 14     |                   | F   | 1.30             | 8       | 0                   |
| 15     |                   | M   | 1.60             | 171     | 1                   |
| 16     |                   | M   | 1.10             | 143     | 5                   |
| 17     |                   | F   | 1.60             | 18      | 0                   |
| 18     |                   | M   | 1.01             | 5       | 0                   |
| 19     |                   | F   | 1.26             | 2       | 0                   |
| 20     |                   | F   | 1.13             | 92      | 0                   |
| 21     |                   | F   | 1.65             | 369     | 3                   |
| 22     |                   | F   | 1.55             | 131     | 0                   |
| 23     |                   | F   | 1.45             | 328     | 2                   |
| 24     |                   | F   | 1.44             | 80      | 2                   |
| 25     |                   | F   | 1.70             | 243     | 0                   |
| 26     |                   | M   | 1.19             | 198     | 1                   |
| 27     |                   | M   | 1.61             | 6       | 1                   |
| 28     |                   | M   | 1.71             | 109     | 1                   |
| 29     |                   | M   | 1.55             | 94      | 1                   |
| 30     |                   | F   | 1.50             | 264     | 3                   |
| 31     |                   | F   | 1.55             | 8       | 0                   |
| 32     |                   | F   | 1.07             | 4       | 0                   |
| 33     |                   | M   | 1.28             | 21      | 0                   |
| 34     |                   | M   | 1.95             | 91      | 1                   |
| 35     |                   | F   | 1.21             | 448     | 4                   |
| 36     |                   | M   | 1.39             | 146     | 1                   |
| 37     |                   | F   | 1.18             | 37      | 1                   |
| 38     |                   | M   | 1.50             | 69      | 0                   |
| 39     |                   | M   | 1.79             | 129     | 0                   |
| 40     |                   | F   | 1.62             | 10      | 0                   |

*A. ziphidarum* [DQ116430], *A. physeteris* [DQ116432], *A. brevispiculata* [DQ116433], *A. paggiae* [DQ116434] and *A. nascettii* [DQ116431], reported in Mattiucci et al. (2009).

## 2.6. Statistical methods

In this study, prevalence and mean intensity of Anisakidae larvae were determined according to these definitions: Prevalence (P) is the number of specimens infected by at least one Anisakidae larva divided by the number of specimens examined. Mean intensity (MI) is the total number of Anisakidae larvae in the sample divided by the number of specimens infected with Anisakidae larvae. Descriptive statistics, Fisher's exact test for comparing P and Bootstrap two-samples *t*-test for comparing mean intensity were performed by QP 3.0 software version 2.0 (Rözsä et al., 2000).

## 3. Results and discussion

The mean weight and standard deviation (SD) of 40 European sea bass were respectively 1.45 ± 0.08 kg for whole fishes and 1.29 ± 0.22 kg for gutted fishes. The fish sex, the number of larvae and muscle

**Fig. 1.** Alignment of the mtDNA *cox2* (629 bp) sequence of the *A. simplex sensu stricto* isolated from *Dicentrarchus labrax* (code *A. simplex* (s.s.) DL) with respect to the other *Anisakis* spp. which have previously been sequenced and deposited in GenBank ((*A. simplex* (s.s.) [DQ116426]; *A. pegreffii* [DQ116428]; *A. simplex C* [DQ116429]; *A. typica* [DQ116427]; *A. ziphidarum* [DQ116430]; *A. nascettii* [DQ116431]; *A. paggiae* [DQ116434]; *A. brevispiculata* [DQ116433]; *A. physeteris* [DQ116432]), by using BioEdit Hall, 1999). Dots indicate identity.

10 20 30 40 50 60 70 80 90 100

A. simplex s.s. DQ116426 TTTTCTAGTTATATAGATTGGTTTCATAACTTTAATTGTAGTTTGCTTTTGGTGTTCCTTTCTTTGTTTCTGTTATGTTTGGTTATCTTCTTTTAGAA  
A. simplex s.s. (DL) .....C.....  
A. pegreffii DQ116428 .....C.....  
A. simplex C DQ116429 .....C.....  
A. typica DQ116427 .....T.....CT.G.....C.....TA..CT.G.....C.....  
A. ziphidarum DQ116430 .....T.....T.A.....A...T...T.A.....G.....  
A. nascettii DQ116431 .....T.....GT.A.A.....T...T.A.....C.....  
A. physeteris DQ116432 .....T.....G..A.....AT.GG.....A.G...A...T...T.A.....AT.....  
A. brevispiculata DQ116433 .....GC.T.....G.A.....GT.AG.....A.A...A...T...T.A.....AT.....  
A. paggiae DQ116434 .....GT.....G.....G.GT.GG.....A...T...T.A.....AT.....

110 120 130 140 150 160 170 180 190 200

A. simplex s.s. DQ116426 ATTTTATTTTAAAGAGTAAGAAGATTGAATATCAGTTTGGTGAACCTTTATGTAGTATTTTCCCTACTTTAATTTTGGTGTGCTCAGATGGTGCCTTCTTT  
A. simplex s.s. (DL) .....  
A. pegreffii DQ116428 .....A.....G.....  
A. simplex C DQ116429 .....C.....A.....G.....  
A. typica DQ116427 .....G.....G.....C..G.....C..G..C...A...C.....  
A. ziphidarum DQ116430 .....G.....A.....G.....C.....C.T.....G..C..A.....  
A. nascettii DQ116431 .....G.....G..A.....G.....G..C.....G..C..A.....C.....  
A. physeteris DQ116432 .....G.....A..A.....G.....G.G.....G.....A.....  
A. brevispiculata DQ116433 .....GG.....A.....A.....G.....G.....C.T.....G.C.....G..C..A.....A..A..A.....  
A. paggiae DQ116434 .....G.....A.....A.....G..C.....GTT.....T.....

210 220 230 240 250 260 270 280 290 300

A. simplex s.s. DQ116426 GAGTTTACTTTATTATTATGGTTTGATGAACTCTTGATAGTAATTTGACTGTGAAAGTTACTGGTCATCAGTGGTATTGGAGTTATGAGTTTAGTGATATC  
A. simplex s.s. (DL) ...C.....C.....A.....  
A. pegreffii DQ116428 .....A...T.....G.....  
A. simplex C DQ116429 .....C.....C.....C.A.....A...A.....G..C.....C.....G...T.....  
A. typica DQ116427 ...A..G.....A..A.....A...C..G...A..C..C.....A.....  
A. ziphidarum DQ116430 .....A...G.....A...T.G.....T.....A..A.....T.....  
A. nascettii DQ116431 .....A...G.....A...T.G.....A..G..A..G..A...G.....A.....A.....T.....  
A. physeteris DQ116432 .....A..AC.TT.G.....C.T.....C.T...T.G.....A.....A..A.....A...A...T.....  
A. brevispiculata DQ116433 .....A..AC.T...C.....G.C.....T...A.....A..A.....A..G.....T.....  
A. paggiae DQ116434 .....A...C.T...C.....A..A...T.G...A..C..A...T.....A..A.....A.....T.....

310 320 330 340 350 360 370 380 390 400

A. simplex s.s. DQ116426 CCGGGTTTAGAATTTGATTCTTATATGAAGTCTGTGGATCAGTTGGAGCTAGGTGAGCCTCGTCTTTTGGAGTTGATAATCGTTGTGTGTTCTTCTGTG  
A. simplex s.s. (DL) .....C.....C.....  
A. pegreffii DQ116428 .....C.....C.....  
A. simplex C DQ116429 .....T...G.....A.....A..T.....  
A. typica DQ116427 .....T..G...G.....T.....A..T.G.....G.....C.....G.....  
A. ziphidarum DQ116430 .....T...G..G.....A.....T.G..A.....C.....GC.T...G.....A.....  
A. nascettii DQ116431 .....C..A..G..G.....A.....T.....A...T.G..C...C..T.G...A.....C.....  
A. physeteris DQ116432 .....T.....A...T.G..A.....A.....A.....G.....  
A. brevispiculata DQ116433 .....T..GC...G.....A..A..G.....C...T.G.....A.....A.....G.....G.....  
A. paggiae DQ116434 .....C.....C.....A.....A.....T.....T...A...A.....AC.A.....G.....G.....

410 420 430 440 450 460 470 480 490 500

A. simplex s.s. DQ116426 ATATTAATGTCCGTTTTTGATTACCTCGGGGGATGTTATTTCATTCTTGGGCTTTGCCTAGAATATCTATTAAGTTGGATGCTATAAGGGGTATTTTGTG  
A. simplex s.s. (DL) .....  
A. pegreffii DQ116428 .....T.....T.....G..G.....C..G..T.....A..  
A. simplex C DQ116429 .....G.....T..T...C.....G.....G.....  
A. typica DQ116427 .....C...A.T...C...C...T.....C.....T..G.....C.....G..CC.T..  
A. ziphidarum DQ116430 .....A.....T..T.....C.....G..G.....A.....G..A.....  
A. nascettii DQ116431 .....A..T.....T..C.....C.....G.....G..A.....C..A..  
A. physeteris DQ116432 .....A...CA.T...T...T...A.....G.....AC.T...G.....  
A. brevispiculata DQ116433 .....A..T.....T..C...A.....A..C..G..G.....AC.T...A.....  
A. paggiae DQ116434 .....A..T.....C.....T..T..C.....T..G.....AC.T...A.....A.....

510 520 530 540 550 560 570 580 590 600

A. simplex s.s. DQ116426 TACTGTTTCTTATAGTTTTCCTACTGTTGGTGTGTTTTATGGTCAATGTTTCAGAGATTTGTGGGGCTAACCATAGTTTTATGCCTATTGCTTTGGAAGTG  
A. simplex s.s. (DL) .....C.....G...  
A. pegreffii DQ116428 .....A.....C.....  
A. simplex C DQ116429 .....C.A.....T.....A.....T...A.....  
A. typica DQ116427 .....C.A.....G..A..T...C..G.....T..A.....T.....A..G..A..  
A. ziphidarum DQ116430 .....T.G.....G..G..T.....G..C..G...C.....T.....C.....A..G..  
A. nascettii DQ116431 .....AT.A...C.....G...T.....G..C.....T...T.....G..T..  
A. physeteris DQ116432 .....T.A.....A.....G..G..T.....T.....T.....A.....A..  
A. brevispiculata DQ116433 .....T.G.....C...A.....G..G..T.....T..A.....T.....A.....G..A..  
A. paggiae DQ116434 .....T.G.....A.....T.....T..A.....A.....A..G..A..  
A. brevispiculata DQ116433 .....C.....C.T.....  
A. paggiae DQ116434 .....C..C.T.....

610 620

A. simplex s.s. DQ116426 ACTTTATTGGATAATTTTAAAGAGTTGGTG  
A. simplex s.s. (DL) .....  
A. pegreffii DQ116428 .....G.....  
A. simplex C DQ116429 .....GC.....  
A. typica DQ116427 .....GC.T.....  
A. ziphidarum DQ116430 .....GC.T.....  
A. nascettii DQ116431 .....GC.T.....  
A. physeteris DQ116432 .....C.TC.T.....  
A. brevispiculata DQ116433 .....C.T.....  
A. paggiae DQ116434 .....C..C.T.....

localisation are reported in Table 1. The sample size of 40 specimens was chosen as the best compromise between a suitable estimate of parasitological indices and the sample costs. In fact, a systematic underestimation of mean intensity was demonstrated when a small sample size was considered (<40) (Marques and Cabral, 2007), whilst, in metazoan parasitology, the P is a variable less affected by sample size than mean intensity, and a sample size of more than 15 specimens is enough to get a good estimate of P (Jovani and Tella, 2006). In accord with Rózsa et al. (2000), the mean abundance was not reported because it is a variable that carries information partially in common with P and MI and its confidence interval (CI) is less precise and informative than MI confidence interval. The digestion method was adopted because it is a very sensitive and accurate method to recover nematode larvae, as reported by Jackson et al. (1981).

The anisakid larvae recovered from viscera and subjected to morphological study were identified as *Anisakis* sp. Type I. The other visceral larvae were genetically identified as *A. simplex* (s.s.) by allozymes according to the allozymes diagnostic for differentiating the species of *Anisakis* spp., as reported in Mattiucci and Nascetti (2006). These results were confirmed by the mtDNA analysis of the *cox2* gene, carried out on some specimens amongst those that had been examined by allozyme markers. The sequences obtained matched 99% with the sequence of the species *A. simplex* (s.s.) deposited in GenBank (Fig. 1).

The visceral P was 0.950 with a 95% CI between 0.829 and 0.991 (determined with Sterne's exact method, adjusted Wald method); the MI was 96.39 with a 95% CI of 67.95–137.58 (determined with Bootstrap). The P of *A. simplex* (s.s.) larvae in muscle was 0.425 with a 95% CI between 0.277 and 0.588; the MI was 1.94 with a 95% CI between 1.41 and 2.59. There is no significant difference between the sexes in the P ( $p=0.200$ ) and MI ( $p=0.171$ ). The results show that P and MI are higher in viscera than in muscle. A visceral P higher than in a previous work (Bernardi, 2009) was found with a significantly high difference ( $p<0.01$ ). This finding is probably bound to the different method used to detect the larvae, in fact in the preliminary study the presence of Anisakidae larvae was detected by visual inspection of viscera whilst in the present study the digestion method was adopted, permitting a more efficient recovery of larvae. This confirms that the observation by eye is a poor method for detecting worms, since the small Anisakidae larvae may be easily missed, although in the viscera of European sea bass they are frequently found covered with a reddish capsule. The host forms a capsule, a stratified squamous epithelium (Berland, 1998), around the larvae to confine them and to prevent their further migration. In mackerel, Jung et al. (2000) isolated an apoptosis-inducing protein (AIP) from the capsules surrounding larval nematode; in authors opinion the AIP expression in capsules is a defensive host mechanism, able to suppress the vitality and ability to invade host tissues. This defensive system may explain the great difference in intensity between viscera and muscle.

Most of the larvae found in muscle were in the belly muscle, only one larva was found in the dorsal fillet. Although digestion method seems to be more efficient to recover larvae from viscera, no additional worms were found in the muscle fillets, perhaps because the fillets was sufficiently thin, which allow an efficient visual inspection. The lack of records about the presence of nematode larvae in the muscle of European sea bass did not permit a statistical comparison of the P and MI. A comparison amongst valuable commercial fishes is important for risk assessment of anisakiasis: in a recent study performed in Scotland waters, Petrie et al. (2005) reported, respectively, the P and MI of *Anisakis* spp., in edible parts, in monkfish (*Lophius budegassa* and *Lophius piscatorius* – 26.96% and 1.70), in cod (*Gadus morhua* – 50.3% and 6.49), in mackerel (*Scomber scomber* – 22.9% and 4.0) and in herring (*Clupea harengus* – 36.0% and 1.85). As found in the present study, *Anisakis* spp. in European sea bass proved a higher P than in almost fish species examined previously by Petrie et al. (2005), except cod and a MI value similar to monkfish and herring.

The findings of such a high P in a smaller size commercial category (1–2 kg) may suggest a greater risk in higher commercial weight classes. In fact the heavy infestation generally observed in the larger fish is also due to the cumulative effect of parasitized preys, since the larvae are able to re-establishing, without further development, in predators.

All the larvae isolated from muscle were identified as *A. simplex* (s.s.); this represents a new host record for the species in Northeast Atlantic waters. Moreover, this finding has a deep impact in anisakiasis prevention, since this species is considered the major etiological agent of anisakiasis in Japan. Umehara et al. (2007) published the molecular identification of one hundred larvae recovered from eighty five patients with anisakiasis; the 98.8% of patients were parasitized by *A. simplex* (s.s.), only one patient was infected by *A. pegreffii*.

In Italy the most frequent species recovered from the patients with anisakiasis is *A. pegreffii* (D'Amelio et al., 1999; Fumarola et al., 2009; Mattiucci et al., 2007, 2011; Moschella et al., 2004), indicating both that this species is widespread in fish species of Italian seas, and that the consumption of raw and/or marinated fish is becoming relatively common.

On this basis it is to be recommended to attend strictly to the requirements concerning parasites provided for by the EC Regulation 853/2004: fishery products to be consumed raw or almost raw must be frozen at a temperature of not more than  $-20^{\circ}\text{C}$  in all parts of the product for not less than 24 h. This treatment assures the anisakid larvae's death, avoiding the possibility of the invasion by larvae in humans. Considering that the main muscle localisation (16 on 17 positive samples) was the belly flaps, these should be removed from European sea bass fillets before sale or processing to remove a large proportion of worms. Unfortunately, freezing and cooking do not assure to solve any health problem related to *Anisakis* presence, because of possible allergic reactions due to antigens which are resistant (Rodríguez-Mahillo et al., 2010; Sanchez-Monsalvez et al., 2006). The risk of allergic reactions after parasitised fish ingestion is a matter of some concern. Some authors reported that sensitised subjects did not show any symptoms after administration of dead larvae (Sastre et al., 2000) on the contrary, other researchers showed that 13 of 64 subjects with *Anisakis* sensitization history had adverse reaction after eating properly cooked fish (Moneo et al., 2007). In the latter study the authors believe that the intolerance recorded could be due to the high amounts of allergens. The presence of *Anisakis* antigens in frozen, cooked and canned fish muscle led to study the effects of high hydrostatic pressure (HHP) on parasitized fish fillets; but the *Anisakis* antigens maintained their allergenic properties, even if the HHP treatment kills the nematode larvae (Vidacek et al., 2009). At present, no treatment can assure safety against allergic adverse reactions, so, patients sensitised to *A. simplex* should be warned of the risk and advised to keep off wild marine fish and preferring farmed fish, where *A. simplex* is not present, as recently verified in farmed European sea bass (Peñalver et al., 2010) or freshwater fish. The present study provides the first occurrence data of *A. simplex* (s.s.) larvae in European sea bass caught in F.A.O. area 27. Further studies on this species from different fishing areas will be necessary to improve the knowledge on epidemiology of this zoonotic parasite.

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