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Title

Anisakis and *Hysterothylacium* species in Mediterranean and North-East Atlantic fishes commonly consumed in Spain: epidemiological, molecular and morphometric discriminant analysis

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Abbreviations: RFLP: restriction fragment length polymorphism; ITS: Internal Transcribed Spacer; GLM: Generalized Linear Model

Abstract

The consumption of raw fish parasitized with larval ascaridoid nematodes of the family *Anisakidae* can cause anisakiasis, provoking gastrointestinal and/or allergic symptomatology. The main causative agents in the *Anisakis* genus are the sibling species *Anisakis simplex* sensu stricto (s.s.) and *A. pegreffii* of the *A. simplex* sensu lato (s.l.) complex. Larvae of *A. simplex* (s.l.) are frequently detected in fish commonly consumed in Spain, as are larvae of the genus *Hysterothylacium* of the family *Raphidascarididae*, associated with allergic reactions but not considered pathogenic. Reported here are the results of an epidemiological survey of ascaridoid larvae in three commonly consumed fish species in Spain, blue whiting (*Micromesistius poutassou*) (n=93), horse mackerel (*Trachurus trachurus*) (n=52) and anchovy (*Engraulis encrasicolus*) (n=69), caught in the North-Eastern Atlantic, West Mediterranean and Adriatic Sea. The larvae found in the dissected fish were identified in the following order of abundance: *A. simplex* (s.l.) (n=2003), *Hysterothylacium aduncum* (n=422), *H. fabri* (n=180) and *A. physeteris* (n=15). Binomial regression analysis showed a correlation between *A. simplex* (s.l.) and *Hysterothylacium* larvae abundance and the host geographical location, the North-Eastern Atlantic being the area with the highest parasitation. Fish length and weight and Fulton's condition factor were correlated with *A. simplex* (s.l.) abundance only in horse mackerel. There was a significant presence of *A. simplex* (s.l.) and *H. aduncum* larvae in the musculature of North-Eastern Atlantic blue whiting, the most parasitized part being the anteroventral region, followed equally by the anterodorsal and central sections. The ITS rDNA of larvae of the sibling species *A. simplex* (s.s.) and *A. pegreffii* was identified by PCR-RFLP, and a binary logistic regression model was developed to study their morphometric differentiation. *Anisakis simplex* (s.s.) was detected in the North-Eastern Atlantic and *A. pegreffii* in all the areas

studied. The morphometric analysis discriminated between the two species at the third and fourth larval stages (L3 and L4), the latter obtained by *in vitro* culture in RPMI-1640 medium. Two discriminant functions were obtained for the L3 and L4 larvae, the ventricle being a key parameter for specific differentiation in both stages, providing taxonomical criteria that could be used besides molecular identification. The present study reveals differences in the parasitation of the studied fish, including the distribution of larvae in the musculature, related to the host species and its geographical origin.

Keywords

Ascaridoid nematodes; larvae identification; morphometry; fish musculature; geographical origin.

1. Introduction

Consuming raw or undercooked marine fish and cephalopods parasitized with larval ascaridoid nematodes of the family *Anisakidae* can cause anisakiasis, provoking severe gastrointestinal and/or allergic symptoms (Audicana et al., 2002; Mattiucci and D'Amelio, 2014). Humans act as accidental hosts in the life cycle of these parasites. According to the European Food Safety Authority (EFSA, 2010), anisakid nematodes constitute the most important 'biological hazard' in seafood products, species of *Anisakis* and *Pseudoterranova* being the main etiological agents of the disease (Mattiucci and Nascetti, 2008). The genus *Hysterothylacium* of the family *Raphidascarididae*, is considered less pathogenic, and is associated with allergic reactions as well as infection after raw fish ingestion (Fernández-Caldas et al., 1998; Valero et al., 2003; Yagi et al., 1996).

Species of *Anisakis*, mainly of the complex *Anisakis simplex* sensu lato (s.l.), and *Hysterothylacium* have been detected in a variety of regularly consumed fishes caught in Spanish marine waters, including horse mackerel, Atlantic mackerel, European hake, blue whiting and European anchovy (Cipriani et al., 2015; Fernández et al., 2005; Gutiérrez-Galindo et al., 2010; MacKenzie et al., 2008; Rello et al., 2009; Roca-Geronès et al., 2018). The frequent consumption of anchovies in vinegar and marinated horse mackerel, together with the growing popularity of exotic raw fish-based dishes such as sushi or ceviche, may represent a health risk for consumers in Spain. More epidemiological information is needed on the extent of infection in fish species involved in the parasite transmission, the spatial distribution of larvae within the fish, especially in the musculature of middle and large specimens, as well as the influence of intrinsic and extrinsic factors of the hosts (Angelucci et al., 2011; EFSA, 2010; Mattiucci et al., 2017).

Anisakis simplex sensu stricto (s.s) and *Anisakis pegreffii*, sibling species of the *A. simplex* (s.l.) complex, are responsible for the majority of human clinical cases of anisakiasis reported worldwide (Mattiucci et al., 2018; Umehara et al., 2007). Reliable identification of *A. simplex* (s.s) and *A. pegreffii* can be achieved with molecular analysis (Mattiucci and Nascetti, 2008; Umehara et al., 2007) however, some authors suggest that morphological characterization of larvae, based mainly on the ventricle length and the ratio between the oesophagus and ventricle length, can differentiate both sibling species (Quiazon et al., 2008).

In the genus *Hysterothylacium*, which includes at least 67 species, the association of larvae with adult forms is made challenging by a high morphological variability. Eighteen larval morphotypes have been described worldwide, and some larvae have been molecularly characterised and associated with adult forms (Hossen and Shamsi, 2019; Roca-Geronès et al., 2018; Shamsi et al., 2013).

The aim of the present work was to survey three commonly consumed fishes from distinct geographical locations for the presence of *Anisakis* and *Hysterothylacium* species and their larval spatial distribution, as well as to determine the main intrinsic and extrinsic factors affecting nematode abundance. Special emphasis was placed on finding larval morphometric discriminant characters that could serve as taxonomical criteria to distinguish the sibling species *A. simplex* (s.s.) and *A. pegreffii*.

2. Materials and methods

2.1 Fish samples and morphological identification of ascaridoid larvae

The parasitological study was performed on three teleostean fishes commonly consumed in Spain, horse mackerel (n = 52) (*T. trachurus*) (Perciformes, Carangidae), blue whiting (n = 93) (*M. poutassou*) (Gadiformes, Gadidae) and anchovy (n = 69) (*E.*

encrasicolus) (Clupeiformes, Engraulidae). Mackerel and whiting were caught in the North-East Atlantic (Iberian coasts) and West Mediterranean (Catalan coasts), corresponding to the FAO (Food and Agriculture Organization of the United Nations) fishing areas 27.8 and 37.1.1, respectively, and the anchovies in the Western Mediterranean and the Adriatic Sea (FAO area 37.2.1). Convenience sampling was carried out, acquiring fish specimens in food markets in Barcelona (Spain) during 2015-17. The selected fish were caught off the Catalan and Northern Spanish coasts, the usual origin of commonly consumed fish. After measuring the total length and weight, the fish were dissected, and the viscera isolated in 9‰ physiological saline solution and examined under a stereomicroscope for the presence of nematode larvae. For all horse mackerel and blue whiting specimens, fish musculature was divided into four sections: the anteroventral muscle or belly flaps (AV), the anterodorsal part (AD), the central dorsoventral part (C) and the posterior part or tail (P); AV musculature was analysed when fresh and the other three parts were frozen at -20ºC for subsequent artificial digestion. Muscle samples of 15-20 g were subjected to a pepsin HCL digestion (1.5 g pepsin, 1:10000 NF) at 37ºC with magnetic stirring for 30-45 minutes. Musculature of anchovies was analysed when fresh. In all cases, the final product was examined in Petri dishes under a stereomicroscope and larvae were isolated and counted. All larvae were fixed and preserved in 70º ethanol. After measuring the total length, specimens were cut in three portions; the central part was reserved for molecular identification, and the anterior and posterior parts were cleared and mounted in lactophenol between the glass slide and coverslip for study under the light microscope. Nematodes were morphologically identified as third-stage larvae (L3) of *Anisakis* and third and fourth-stage larvae (L4) of *Hysterothylacium* species. Characterization was mainly based on the presence/absence of an apical tooth and lips, the anatomy of the anterior part of the

digestive tract, the presence/absence of a mucron, the caudal end anatomy and the position of the excretory pore (Berland, 1961; Petter and Maillard, 1988). Larvae of *Hysterothylacium* were also described as larval types or morphotypes, following the criteria of Shamsi et al. (2013).

2.2 In vitro culture of Anisakis larvae

A selection of 100 undamaged L3 larvae of *A. simplex* (s.l.), isolated from blue whiting of the North-East Atlantic, were cultured *in vitro* to obtain L4 larvae for the morphometric study. Larvae were firstly washed and axenized in 24-well sterile polystyrene tissue-culture plates with an antibiotic and antifungal solution (Iglesias et al., 1997) and subsequently cultured in commercial medium RPMI-1640 + 20% (v/v) FBS (foetal bovine serum) + 0.1% w/v pepsin (1:10000 NF), adjusting the pH to 4.0 as reported by Iglesias et al. (2001). Culture was performed in an incubator at 37°C provided with an atmosphere of 5% CO2. Culture plates were observed every 24 hours under the inverted microscope to monitor larval evolution.

2.3 Molecular identification of Anisakis species by PCR-RFLP

The specific identification of *Anisakis* larvae was carried out using the molecular technique PCR-RFLP (restriction fragment length polymorphism). Genomic DNA was isolated using the Pure PCR Template Extraction Kit (Roche), according to the manufacturer's protocol. The ITS region was targeted for PCR amplification as described by D'Amelio et al. (2000). The entire ITS region (ITS1, 5.8S and ITS2) was amplified using NC5 and NC2 primers. The PCR was performed in a PTC-200 DNA motor thermocycler (MJ Researcher) with a hybridization temperature of 55º C. DNA amplification products were digested with restriction endonucleases *Hinf*I and *Taq*I

(New England Biolabs) at 37º C for 90 min. The digested products were subjected to electrophoresis in 2% agarose gel (wide range/standard 3:1 - Sigma A7431) and visualized by a UV light Illuminator 2000 (BIO-Rad).

2.4 Morphometric discriminant analysis

The L3 and L4 larvae, the latter obtained by *in vitro* culture, of the sibling species *A. simplex* (s.s.) and *A. pegreffii* underwent morphometric analysis for discriminant factors. The following morphological parameters were taken into account for both larval stages: body length and width, distance from the nerve ring to the anterior end, oesophagus length and width, ventricle length and width, and tail length and width.

In order to obtain a mathematical function to infer morphological discrimination between the two *Anisakis* species in both L3 and L4 larvae, the morphometric data were analysed using a Generalized Linear Model (GLM). The specific molecular identification by PCR-RFLP of each larva was transformed into a binary response variable; morphometric measurements were considered as predictor variables, and fish species and geographical origin as categorical factors. The analysis was carried out by means of a binary logistic regression, using a logit link function in the SPSS v22 software. The minimal acceptable model was derived by backward stepwise deletion from a preliminary model that included the main effects and interactions of all morphometric variables. Each variable was analysed independently to test its influence in the overall significance of the model, taking into account Wald's coefficient (B). The cut-off for keeping a variable in the final model, establishing the level of significance, was set to $p<0.05$ (Garson, 2013).

2.5 Epidemiological concepts and statistical analysis

Estimated prevalence, intensity and abundance, as descriptive epidemiological terms (Bush et al., 1997), were calculated using the Quantitative Parasitology Software 3.0 (Rózsa et al., 2000), as well as the dispersion index or variance to mean abundance ratio (var/mean), in the 95% confidence interval (CI) for each parameter (2000 bootstrap replications). To validate the presence/absence of the nematode species and the distribution of abundance, randomized models were used to test differences in the epidemiological data for each nematode and host species (Lotz and Font, 1994). The nonparametric Chi-square (χ^2) and Mann-Whitney U statistics (U) were used to assess differences in prevalence and mean intensity/abundance, respectively.

The abundance of the nematode species in their hosts was analysed in relation to the fish length (L) and weight (W) and Fulton's condition factor (K) as intrinsic variables, and the geographical origin as an extrinsic variable. Fulton's condition factor, calculated from the fish length and weight ($K = 100*(W/L^3)$), is an indicator of the physiological condition of the fish, which is optimal when $K \geq 1$ (Ricker, 1975). A GLM analysis using a negative binomial regression (1000 bootstrap replications) was applied to obtain a significant model to study the influence of these intrinsic and extrinsic variables on the abundance of nematode species, which was considered as a dependent variable. In all cases, the minimal acceptable model was derived by backward stepwise deletion, as described in section 2.4 (Garson, 2013). Spearman's Rho (Rho) nonparametric test was applied to analyse the correlation between larval abundance and the fish intrinsic variables with significant influence in the abundance model. All parameters and variables were tabulated and in some cases recoded for their analysis in SPSS v22 software.

3. Results

3.1 Host parasitic infection levels by Anisakis and Hysterothylacium species

The parasitological analysis of 214 specimens of horse mackerel (*T. trachurus*), blue whiting (*M. poutassou*) and anchovy (*E. encrasicolus*) resulted in the detection of 2621 ascaridoid larvae, which were morphologically identified as different species of *Anisakis* and *Hysterothylacium*. Among the *Anisakis* L3 larvae, *A. simplex* (s.l.) was the most abundant species, with up to 2003 larvae, while 15 larvae were identified as *A. physeteris*. Regarding the genus *Hysterothylacium*, 421 L3 and 1 L4 larvae were identified as *H. aduncum*, and 1 L3 and 179 L4 larvae as *H. fabri*. The specific identification of *Hysterothylacium* larvae was carried out by morphotype and genotype characterization in a previous study using the same groups of larvae as here (Roca-Geronès et al., 2018). Morphotypes VIII and IX corresponded to L3 and L4 larvae, respectively, and shared the *H. aduncum* genotype; morphotypes III and IV corresponded to L3 and L4 larvae, respectively, and shared the *H. fabri* genotype, in accordance with the study by Tedesco et al. (2018). *Anisakis* and *Hysterothylacium* coinfection occurred in 46.3% of parasitized fish of the three host species.

The results of the analysis of the descriptive epidemiological variables of host parasitation by ascaridoid species, as well as intrinsic characteristics of the fish, are given in Table 1. The highest prevalence and abundance of *A. simplex* (s.l.) were detected in North-Eastern Atlantic horse mackerel and blue whiting, with values significantly higher than in their congeners in the Western Mediterranean ($p<0.01$). In anchovies, *A. simplex* (s.l.) was detected in specimens of the Adriatic but not the Western Mediterranean ($p<0.01$). A low intensity of *A. physeteris* larvae was found in one horse mackerel and four blue whiting of the Western Mediterranean, and one blue whiting of the North-Eastern Atlantic. In the genus *Hysterothylacium*, *H. aduncum* was detected in all host species and geographical areas studied, with higher prevalence and

abundance in the North-Eastern Atlantic than in the Western Mediterranean ($p<0.01$). No significant differences were found for this raphidascaridid between anchovies of the Western Mediterranean and Adriatic. *H. fabri* was detected only in the Western Mediterranean, mainly in horse mackerel, showing a significantly higher prevalence than *H. aduncum* in the same host and area ($p<0.01$).

Almost all species of nematode exhibited an aggregated distribution (var/mean >1) in their hosts. *A. simplex* (s.l.) in North-Eastern Atlantic blue whiting (var/mean=133.7) stands out with three specimens containing over 100 larvae. Exceptionally, *H. aduncum* and *H. fabri* displayed a non-aggregated distribution (var/mean ≤ 1) in Western Mediterranean blue whiting.

3.2 Host factors influencing larval abundance

Significant predictive models were obtained to analyse the influence of different intrinsic and extrinsic variables of the host species on nematode species abundance. Geographical origin proved to be a significant variable for *A. simplex* (s.l.), being a key factor in predicting the abundance of this nematode in both horse mackerel and blue whiting ($p<0.01$). The length and weight of the fish contributed significantly to the horse mackerel model ($p<0.01$ and $p<0.05$, respectively), in which *A. simplex* (s.l.) abundance was inversely proportional to Fulton's condition factor in both Atlantic and Mediterranean specimens ($p<0.01$).

Regarding *H. aduncum*, geographical origin ($p<0.01$), host length ($p<0.05$), host weight ($p<0.05$) and Fulton's condition factor ($p<0.05$) contributed significantly to the model of abundance in blue whiting. Given the restrictive global distribution of *Hysterothylacium* species in horse mackerel (Table 1), the geographical origin was discarded for the analysis of *H. aduncum* abundance. In Atlantic specimens of the same

fish species, host length had a notable influence on *H. aduncum* abundance, also showing a significant correlation ($p<0.01$). Significant variables in the *H. fabri* abundance model in Western Mediterranean host mackerel were host length, host weight and Fulton's condition factor ($p<0.01$).

3.3 Spatial distribution of larvae in fish

Nematode larvae were mainly localized in the visceral cavity, either free or encapsulated around the visceral organs. Notably, however, *A. simplex* (s.l.) and *H. aduncum* were found in the edible part of fish. Prevalence, intensity and abundance of ascaridoid species in the musculature are shown in Table 2. In horse mackerel and blue whiting, the presence of *A. simplex* (s.l.) in the musculature was significantly higher in Atlantic than in Western Mediterranean specimens ($p<0.01$). With respect to *H. aduncum*, this divergence between Atlantic and Mediterranean hosts was only observed in blue whiting ($p<0.01$). In anchovies, significant differences were detected for *A. simplex* (s.l.) between Western Mediterranean and Adriatic specimens ($p<0.01$).

In North-Eastern Atlantic hosts, blue whiting had a markedly higher abundance of *A. simplex* (s.l.) ($p<0.05$) and *H. aduncum* ($p<0.01$) in the musculature than horse mackerel (Fig. 1). The larval distribution pattern in the parasitized blue whiting was similar for the two nematode species, although the parasitic load of *A. simplex* (s.l.) was greater than *H. aduncum* (Fig. 2). The belly flaps or anteroventral region were the most parasitized part of the musculature ($p<0.01$) and the posterior section the least ($p<0.01$ for *A. simplex* (s.l.) and $p<0.05$ for *H. aduncum*). No significant differences were found between the anterodorsal and posterior sections ($p = 0.36$ for *A. simplex* (s.l.) and $p = 0.77$ for *H. aduncum*).

3.4 In vitro culture and morphological identification of Anisakis simplex (s.l.) *larvae*

In vitro culture of L3 larvae of *A. simplex* (s.l.) isolated in blue whiting of the North East Atlantic was 100% successful in obtaining L4 larvae. After 4 days of culture, all the L3 larvae had moulted into L4, whose morphological features included labia instead of the tooth, the absence of a caudal mucron, and signs of the moult in the culture media, as observed by Iglesias et al. (2001).

3.5 Molecular identification of Anisakis species

A selection of larvae morphologically identified as *A. simplex* (s.l.) from all host species and geographical areas was molecularly analysed (Table 3). *A. simplex* (s.s) was identified in North-Eastern Atlantic horse mackerel and blue whiting, while the sibling species *A. pegreffii* was identified in all hosts and areas studied. Coinfection of the two *Anisakis* species was observed in three horse mackerel and five blue whiting specimens from the North-Eastern Atlantic; a few hybrid specimens were also detected in fishes from both the North-Eastern Atlantic and Western Mediterranean. On the other hand, *A. simplex* (s.l.) L4 larvae obtained by *in vitro* culture of L3 larvae from North-Eastern Atlantic blue whiting were identified as *A. simplex* (s.s.) (n=56) and *A. pegreffii* (n=24). The morphological identification of *A. physeteris* larvae was confirmed by PCR-RFLP.

3.6 Morphometric discriminant study of L3/L4 larvae of A. simplex (s.s.*) and A. pegreffii*

For the morphometric study, the measures of morphological variables of L3 and L4 larvae of the two molecularly identified species *A. simplex* (s.s.) and *A. pegreffii* (Table 4) were processed. The GLM analysis showed that none of the studied morphological parameters were individually able to distinguish between the two *Anisakis* species,

based on morphometric measures. However, it was possible to estimate two model equations capable of differentiating between these species for L3 ($p<0.01$) and L4 stages ($p<0.01$), respectively. For L3 larvae, the final model included the main effects of three variables: distance from the nerve ring to the anterior end ($B = 0.027$, $p<0.05$), the oesophagus length/ventricle length ratio ($B = -4.282$, $p<0.01$) and the ventricle length/ventricle width ratio ($B = -2.657$ $p<0.01$). The factors of host species and geographical origin included in the initial model did not show a significant influence. The L4 larval regression model included the main effects of four independent variables: ventricle length ($B = -0.013$, $p<0.05$), ventricle width ($B = 0.089$, $p<0.01$), oesophagus width ($B = -0.104$, $p<0.01$) and body length ($B = 0.393$, $p<0.05$).

The discriminant capacity of both models was tested by contrasting the predictive values obtained when applying the corresponding L3 or L4 model to each larva specifically identified by PCR-RFLP. Predictive and molecular data did not differ significantly (χ^2: 10.08 and 26.24, for L3 and L4 respectively; $p<0.01$), correctly differentiating between *A. simplex* (s.s.) and *A. pegreffii* in 78.4% of L3 and 82.6% of L4 larvae.

4. Discussion

The main species of nematode larvae detected in the studied fish were *A. simplex* (s.l.), *H. aduncum* and *H. fabri*. Their presence varied according to the host species and the fishing area, as described by other authors (Cipriani et al., 2018; Mattiucci et al., 2018; Rello et al., 2009). Among the extrinsic and intrinsic factors studied, geographical origin had the most influence on the abundance of *A. simplex* (s.l.) and *H. aduncum*. Estimated prevalence and abundance of *A. simplex* (s.l.) were significantly higher in horse mackerel and blue whiting from the North-Eastern Atlantic than the Western

Mediterranean. The number of host specimens studied was not high, especially regarding North-East Atlantic horse mackerel, and *A. simplex* (s.l.) prevalence showed near-overlapping confidence intervals between both areas. Nevertheless, the large differences in intensity and abundance are indicative of the distribution of *A. simplex* (s.l.) in different areas, although the results would be more consistent by expanding the sample. A few previous reports on the same host species and FAO fishing areas are available (Adroher et al., 1996; Madrid et al., 2012). *A. simplex* (s.l.) parasitation was not observed in anchovies caught in the Western Mediterranean, in accordance with previous studies (Cipriani et al., 2018; Rello et al., 2009), whereas it was notable in specimens from the Adriatic. It has been suggested that the Adriatic Sea is a hotspot for *Anisakis*, as its special biotic and abiotic conditions favour intermediate and paratenic hosts (Cipriani et al., 2018; Mladineo and Poljak, 2014). Likewise, the detection of *A. simplex* (s.l.) is thought to be directly related to the presence of definitive hosts (Klapper et al., 2015; Mattiucci and Nascetti, 2008; Rello et al., 2009). This would explain the low prevalence of *Anisakis* larvae in the Mediterranean Sea, an enclosed area with a lower number of cetacean definitive hosts than the Atlantic Ocean (Notarbartolo di Sciara, 2002). Thus, the results for fish from the Catalan coast differ from those obtained in other Western Mediterranean locations, such as the Gulf of Lion (France) and the Ligurian Sea (Italy), where the number of cetaceans and the prevalence of *A. simplex* (s.l.) in fish is higher (Rello et al., 2009; Serraca et al., 2013).

Host length has been previously described as a significant factor in the abundance of *A. simplex* (s.l.) in horse mackerel (Abattouy et al., 2014; Shawket et al., 2017; Tantanasi et al., 2012), but not combined with host weight, as in the present study. Also, an inverse direct correlation between *A. simplex* (s.l.) abundance and Fulton's condition factor is reported for the first time in horse mackerel. This correlation has been observed

previously in *Gadus morhua* from the Baltic Sea (Horbowy et al., 2016). In contrast, a positive correlation between Fulton's condition factor and *A. simplex* (s.l.) abundance was observed in *Katsuwonus pelamis* caught off the Madeira Islands, which was associated with a higher feeding rate in the most infected specimens (Hermida et al., 2018). In the current work, only a very low percentage of *A. physeteris* was detected in hosts from both the North-Eastern Atlantic and Western Mediterranean, as observed in previous studies (Mattiucci et al., 2015; Piras et al., 2014; Valero et al., 2000).

Comparison of the data obtained here on *Hysterothylacium* larvae with previous studies is difficult, as few reports mention both *H. aduncum* and *H. fabri* in the fishing areas under study, and prevalence and intensity values differ considerably for the same fish species. (Adroher et al., 1996; Amor et al., 2011; Madrid et al., 2012; Pekmezci et al., 2013; Rello et al., 2009). The high abundance of *H. fabri* in Western Mediterranean horse mackerel could be related to the diet of the host, which would explain why its detection is restricted to the Mediterranean Sea, mainly in horse mackerel (Valero et al., 2006).

Most of the nematode species detected in the present study showed an aggregated distribution within the hosts, which is common in parasite communities and has been previously observed in fish with *Anisakis* (Mladineo et al., 2012; Mladineo and Poljak, 2014). The high degree of aggregation observed, especially in North-Eastern Atlantic fish, indicates that some specimens harboured a particularly high number of parasites in comparison with the rest of the analysed hosts, which could increase the health risk for consumers, particularly if larvae are located in the flesh. In contrast, the distribution of *H. aduncum* and *H. fabri* in Western Mediterranean blue whiting was regular and non-aggregated (var/mean ≤ 1), probably due to the low number of larvae found.

Spatial distribution of larvae in fish visceral organs vs musculature, which seems to depend on the host species (Klapper et al., 2015), is important in terms of public health, as the presence of larvae in edible parts increases the risk for consumers. In the present work, the highest prevalence and abundance of parasitation in fish edible parts was observed in North-Eastern Atlantic blue whiting for both *A. simplex* (s.l.) and *H. aduncum*. This contrasts with the zero or low parasitation in the flesh of the Atlantic horse mackerel, which otherwise showed a high prevalence and abundance of *A. simplex* (s.l.) and *H. aduncum* in non-edible parts. Other authors have also observed more larvae in the flesh of blue whiting than in horse mackerel from the North-Eastern Atlantic (Madrid et al., 2012; Sanmartin Duran et al., 1989). This could be explained by post-mortem larval migration, detected by the same authors in blue whiting, with a significant correlation between the number of *A. simplex* (s.l.) larvae in the musculature and the time elapsed after capture.

The distribution of *A. simplex* (s.l.) larvae in the musculature of blue whiting has only been studied by Gómez-Mateos et al. (2016), who divided the flesh along the ventral/dorsal axis. Studies in hake and other host species from the North-Eastern Atlantic, analysing mainly the ventral/dorsal axis of the fish, detected the ventral region as the most parasitized portion (Cipriani et al., 2015; Klapper et al., 2015; Levsen and Karl, 2014). In the present study, to ascertain the exact localization of larvae in the musculature of the larger fish species, horse mackerel and blue whiting, an anterior region was studied, divided into dorsal and ventral sections, followed by a central and posterior part. The most parasitized was the anteroventral region, or belly flaps, whereas the anterodorsal and central sections were affected to a similar degree, the number of larvae decreasing towards the tail of the fish.

Although blue whiting is not usually consumed raw in Spain, the great abundance of *A. simplex* (s.l.) detected in the belly flaps indicates that thorough cooking is essential to reduce the health risk for consumers. On the other hand, despite containing fewer larvae in the flesh, horse mackerel represents a risk as it is consumed marinated. The main cause of human gastric anisakiasis in Europe, however, are marinated anchovies, Adriatic specimens more so than those of the Western Mediterranean (Mattiucci et al., 2013; Rello et al., 2009).

Regarding *H. aduncum*, this species showed the same pattern of larval distribution in the musculature as *A. simplex* (s.l.) in North-Eastern Atlantic blue whiting, but with lower abundance values. Although generally considered non-pathogenic for humans, this species should be also taken into account when inspecting edible parts for larvae due to its association with allergic reactions (Fernández-Caldas et al., 1998; Valero et al., 2003).

It has been suggested that *A. simplex* (s.s.) and *A. pegreffii* of the *A. simplex* (s.l.) complex have different pathogenic potential (Arizono et al., 2012; Romero et al., 2013), so their specific identification is important in the assessment of health risk. In the same fish hosts as the current study, other authors have also identified *A. simplex* (s.s.) in the North-Eastern Atlantic and *A. pegreffii* in all three studied areas, the North-Eastern Atlantic, Western Mediterranean and Adriatic (Mattiucci et al., 2015; Mattiucci and Nascetti, 2008; Mladineo et al., 2012; Valero et al., 2000). At the larval stage, in contrast with the adult stage, no single morphological feature can differentiate between the sibling species of the *A. simplex* (s.l.) complex (Mattiucci et al., 2018). With the aim of finding an alternative to molecular identification methods, morphometric parameters of specifically identified larvae were statistically analysed. Based on some of the morphometric characters, two discriminant functions were obtained for L3 and L4

larvae, respectively, of *A. simplex* (s.s.) and *A. pegreffii*, without significant differences regarding the results of molecular identification.

Few recent studies have described differential morphometric characters for the larvae of these sibling species. Quiazon et al. (2008), in their study of larvae in fish hosts in the Japanese Sea, defined the ventricle length as a clear morphological parameter distinguishing *A. simplex* (s.s.) from *A. pegreffii* at both L3 and L4 development stages, with support from subsequent studies in Asiatic Pacific coasts (Setyobudi et al., 2011; Zhang et al., 2013). In the present study, the discriminant capacity of the ventricle measurements was not observed, in accordance with a previous analysis of the larvae of the two sibling species in Adriatic fish (Vardić Smrzlić et al., 2012). These discrepant results could indicate morphometric variability among larvae related to geographical origin. Nevertheless, the parameters related to the ventricle can be considered as key variables, because they are included in both of the obtained discriminant functions. The discriminant capacity of the estimated equations did not differ significantly from the results of molecular identification, indicating their utility as an alternative for species-specific identification.

The present study reveals the health risk posed by certain fishes habitually consumed in Spain due to parasitation by *Anisakis* and *Hysteothylacium* larvae. The risk varies according to the host species, the geographical origin, the presence of larvae in the flesh and the mode of consumption. Specific identification of *Anisakis* sibling species is important from an epidemiological point of view but also to know if the pathology is species-related. In this respect, the two functions derived from the data acquired in the present study constitute a useful alternative to molecular methods.

Conflict of interest

The authors declare that they have no conflicts of interests.

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Figure captions

Fig. 1. Boxplots showing the total, visceral and muscular distribution of the larvae of *Anisakis simplex* (s.l.) and *Hysterothylacium aduncum* in *T. trachurus* and *M. poutassou* of the North-Eastern Atlantic.

Fig. 2. Relative larval distribution pattern of *A. simplex* (s.l.) and *H. aduncum* in the different sections of the musculature of the parasitized specimens of North-Eastern Atlantic *M. poutassou*. n: number of total larvae detected.

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Table1

Descriptive epidemiological results of fish infection with larvae of ascaridoid species and intrinsic characteristics of the fish, according to host species and geographical area.

Host				Prevalence‡ % (95% CI)					Mean Intensity (95% CI)				Abundance (95% CI)				Var/mean			
Area	Species	ML (cm) (range)	MW (g) (range)	Total	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*
NEA	TT (n = 19)	30.4 (25.0-34.0)	273.7 (152.4-347.9)	100 (82-100)	100 (82-100)	-	63.2 (39-82)	-	26.6 (16.3-53.1)	-	6.6 (3.8-11.3)	-	26.6 (16-53)	-	4.2 (2-8)	-	48.4	-	8.9	-
	MP (n = 44)	24.4 (18.9-37.0)	96.4 (41.9-229.0)	84.1 (70-92)	79.5 (65-89)	2.3 (0-12)	68.2 (53-81)	-	39.3 (21.9-69.5)	6 (*)	8.6 (6.0-13.0)	-	31.3 (17-56)	0.14 (0-0.5)	5.9 (3.7-9.1)	-	133.7	6.0	14.3	-
WM	TT (n = 33)	27.5† (21.5-33.4)	177.6† (82.2-359.0)	87.9 (72-96)	63.6 (45-79)	3.0 (0-16)	15.2 (6-32)	69.7 (52-84)	3.4 (2.5-4.5)	1 (*)	3.4 (1.6-5.4)	7.8 (4.6-16.6)	2.1 (1.4-2.9)	0.06 (0-0.2)	0.52 (0.12-1.24)	5.4 (3.03-11.8)	2.7	2.0	4.5	22.8
	MP (n = 49)	24.1† (17.5-32.5)	103.8† (36.0-264.3)	34.7 (22-49)	30.6 (19-45)	8.2 (3-19)	6.1 (2-17)	2.0 (0-11)	1.5 (1.1-1.9)	2 (1.0-3.0)	1 (*)	1 (*)	0.45 (0.2-0.7)	0.16 (0.04-0.6)	0.06 (0-0.12)	0.02 (0-0.06)	1.4	3.4	0.96	1.0
	EE (n = 30)	13.3 (9.5-15.0)	18.3 (6.4-25.9)	53.3 (35-70)	-	-	53.3 (35-70)	-	-	-	2.1 (1.4-3.3)	-	-	-	1.1 (0.7-1.9)	-	-	-	2.9	-
AD	EE (n = 39)	14.2† (13.0-16.5)	19.9† (12.2-27.6)	79.5 (64-90)	46.2 (31-62)	-	53.8 (38-69)	-	1.8 (1.4-2.1)	-	1.5 (1.2-1.8)	-	0.85 (0.5-1.2)	-	0.8 (0.5-1.1)	-	1.3	-	1.1	-

NEA: North-East Atlantic; WM: West Mediterranean; AD: Adriatic; TT: *Trachurus trachurus*; MP: *Micromesistius poutassou*; EE: *Engraulis encrasicolus*; ML: mean length; MW: mean weight; *A.s.*(s.l.): *Anisakis simplex* (sensu lato); *A.p.*: *Anisakis physeteris*; *H.a.: Hysterothylacium aduncum; H. f.: Hysterothylacium fabri*; CI: confidence interval; Var/mean: variance to mean ratio; †: number of measured and weighed hosts was 19 for WM TT, 48 for WM MP and 24 for AD EE; ‡: Estimated prevalence; *Only one host infected

Table 2
Parasitation rate for third-stage larvae (L3) *A. simplex* (s.l.) and *H. aduncum* in the musculature of the examined fish and areas.

Area	Host species	*A. simplex* (s.l.)			*H. aduncum*		
		P% (95% CI)	MI (95% CI)	A (95% CI)	P% (95% CI)	MI (95% CI)	A (95% CI)
NEA	*T. trachurus* (n = 19)	36.8 (16.3-61.6)	1.43 *	0.53 (0.17-0.96)	5.3 (0.0-26.0)	2	0.11 (0.00-0.33)
	M. poutassou (n = 44)	59.1 (43.3-73.7)	10.27 (4.38-17.32)	6.07 (2.45-10.98)	50.0 (34.6-65.4)	4.18 (2.72-6.00)	2.09 (1.20-3.19)
WM	*T. trachurus* (n = 33)	0.0 (0.0-10.6)	-	-	3.0 (0.0-15.8)	1 *	-
	M. poutassou (n = 49)	2.0 (0.0-10.9)	1 *		0.0 (0.0-7.3)	-	-
	E. encrasicolus (n = 30)	0.0 (0.0-11.6)	-	-	16.7 (5.6-34.7)	1.4 *	0.23 (0.004-0.48)
AD	*E. encrasicolus* (n = 39)	25.6 (13.0-42.1)	1.1 (1.0-1.4)	0.28 *	17.9 (7.5-33.5)	1.14 (1-1.5)	0.21 (0.08-0.36)

NEA: North-East Atlantic, WM: West Mediterranean, AD: Adriatic, P: prevalence MI: mean intensity A: abundance, CI: Confidence interval, *Not calculable.

Table 3
Specific identification by PCR-RFLP of the ITS rDNA using *Hinf*I restriction enzyme of *Anisakis* third-stage larvae (L3) of hosts and geographical areas studied.

Area	Host species	N	n	*Anisakis simplex* (s.s.)	*Anisakis pegreffii*	Hybrid *A.s.*(s.s.)/*A.p.*	*Anisakis physeteris*
NEA	TT	9	22	10	11	1	-
	MP	10	54	39	8	1	6
WM	TT	11	32	-	28	3	1
	MP	11	19	-	13	-	6
AD	EE	3	5	-	5	-	-
Total		44		49	65	5	13

NEA: North-East Atlantic; WM: West Mediterranean; AD: Adriatic; TT: *Trachurus trachurus*; MP: *Micromesistius poutassou*; EE: *Engraulis encrasicolus*; N: number of studied hosts; n: number of larvae identified

Table 4

Measurements of the main morphological parameters of third and fourth stage larvae of *A. simplex* (s.s.) and *A. pegreffii*.

	Third-stage larvae (L3)						Fourth-stage larvae (L4) (four days cultured larvae)					
	A. simplex (s.s.) (n = 29)			*A. pegreffii* (n = 45)			*A. simplex* (s.s.) (n = 45)			*A. pegreffii* (n = 24)		
	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% Cl	Range
Body length (BL)	17.70	16.63-18.68	13.02-4.0	16.97	16.11-17.82	10.0-24.0	22.98	21.56-24.41	20.5-36.0	22.60	21.11-24.18	16.5-29.0
Body width (BW)	0.45	0.43-0.48	0.27-0.56	0.45	0.43-0.47	0.22-0.56	0.51	0.50-0.52	0.40-0.64	0.51	0.48-0.53	0.35-0.62
NR to anterior end	0.26	0.25-0.27	0.21-0.32	0.26	0.25-0.27	0.22-0.33	0.40	0.39-0.42	0.35-0.45	0.41	0.39-0.42	0.33-0.46
Ventricle length (VL)	0.92	0.88-0.96	0.71-1.15	0.87	0.82-0.91	0.57-1.15	0.94	0.89-0.99	0.81-1.25	0.86	0.80-0.91	0.59-1.13
Ventricle width (VW)	0.25	0.24-0.26	0.19-0.31	0.26	0.25-0.27	0.15-0.33	0.21	0.21-0.22	0.18-0.26	0.25	0.24-0.26	0.19-0.29
Oesophagus length (OL)	1.97	1.86-2.07	1.39-2.45	1.78	1.70-1.86	1.32-2.28	2.64	2.58-2.71	2.25-3.13	2.55	2.44-2.65	1.99-2.94
Oesophagus width (OL)	0.14	0.13-0.15	0.07-0.17	0.14	0.14-0.15	0.09-0.18	0.20	0.19-0.21	0.18-0.25	0.20	0.19-0.21	0.17-0.24
Tail length	0.09	0.08-0.10	0.05-0.13	0.09	0.08-0.09	0.05-0.12	0.14	0.13-0.14	0.10-0.19	0.13	0.12-0.14	0.09-0.19
Tail width	0.13	0.12-0.14	0.10-0.17	0.13	0.13-0.27	0.17-0.05	0.16	0.15-0.17	0.13-0.19	0.15	0.14-0.16	0.13-0.20
OL/BL (%)	11.25	10.69-11.81	8.7-15.4	10.7	10.21-11.09	7.4-13.2	11.87	11.20-12.55	8.2-17.3	11.48	10.69-12.27	9.1-15.5
VL/BL (%)	5.25	5.03-5.48	4.4-6.7	5.2	4.91-5.42	3.4-6.6	4.15	4.00-4.29	3.4-6.0	3.82	3.62-4.01	3.1-5.0
OL/VL	2.14	2.06-2.24	1.6-2.6	2.1	2.02-2.14	1.6-2.4	2.87	2.73 3.01	2.3-4.0	3.02	2.84-3.20	2.1-3.8
VL/VW	3.73	3.57-3.89	2.7-4.6	3.3	3.14-3.50	2.2-4.7	4.46	4.20-4.72	3.6-6.7	3.48	3.24-3.71	2.2-4.8

NR: nerve ring; n: number of measured larvae; CI: confidence interval; range: minim and maxim values; All measurements are given in mm.

Table1

Descriptive epidemiological results of fish infection with larvae of ascaridoid species and intrinsic characteristics of the fish, according to host species and geographical area.

Host				Prevalence‡ % (95% CI)					Mean Intensity (95% CI)				Abundance (95% CI)				Var/mean			
Area	Species	ML (cm) (range)	MW (g) (range)	Total	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*	*A.s.*(s.l.)	*A.p.*	*H.a.*	*H.f.*
NEA	TT (n = 19)	30.4 (25.0-34.0)	273.7 (152.4-347.9)	100 (82-100)	100 (82-100)	-	63.2 (39-82)	-	26.6 (16.3-53.1)	-	6.6 (3.8-11.3)	-	26.6 (16-53)	-	4.2 (2-8)	-	48.4	-	8.9	-
	MP (n = 44)	24.4 (18.9-37.0)	96.4 (41.9-229.0)	84.1 (70-92)	79.5 (65-89)	2.3 (0-12)	68.2 (53-81)	-	39.3 (21.9-69.5)	6 (*)	8.6 (6.0-13.0)	-	31.3 (17-56)	0.14 (0-0.5)	5.9 (3.7-9.1)	-	133.7	6.0	14.3	-
WM	TT (n = 33)	27.5† (21.5-33.4)	177.6† (82.2-359.0)	87.9 (72-96)	63.6 (45-79)	3.0 (0-16)	15.2 (6-32)	69.7 (52-84)	3.4 (2.5-4.5)	1 (*)	3.4 (1.6-5.4)	7.8 (4.6-16.6)	2.1 (1.4-2.9)	0.06 (0-0.2)	0.52 (0.12-1.24)	5.4 (3.03-11.8)	2.7	2.0	4.5	22.8
	MP (n = 49)	24.1† (17.5-32.5)	103.8† (36.0-264.3)	34.7 (22-49)	30.6 (19-45)	8.2 (3-19)	6.1 (2-17)	2.0 (0-11)	1.5 (1.1-1.9)	2 (1.0-3.0)	1 (*)	1 (*)	0.45 (0.2-0.7)	0.16 (0.04-0.6)	0.06 (0-0.12)	0.02 (0-0.06)	1.4	3.4	0.96	1.0
	EE (n = 30)	13.3 (9.5-15.0)	18.3 (6.4-25.9)	53.3 (35-70)	-	-	53.3 (35-70)	-	-	-	2.1 (1.4-3.3)	-	-	-	1.1 (0.7-1.9)	-	-	-	2.9	-
AD	EE (n = 39)	14.2† (13.0-16.5)	19.9† (12.2-27.6)	79.5 (64-90)	46.2 (31-62)	-	53.8 (38-69)	-	1.8 (1.4-2.1)	-	1.5 (1.2-1.8)	-	0.85 (0.5-1.2)	-	0.8 (0.5-1.1)	-	1.3	-	1.1	-

NEA: North-East Atlantic; WM: West Mediterranean; AD: Adriatic; TT: *Trachurus trachurus*; MP: *Micromesistius poutassou*; EE: *Engraulis encrasicolus*; ML: mean length; MW: mean weight; *A.s.*(s.l.): *Anisakis simplex* (sensu lato); *A.p.*: *Anisakis physeteris*; *H.a.: Hysterothylacium aduncum; H. f.: Hysterothylacium fabri*; CI: confidence interval; Var/mean: variance to mean ratio; †: number of measured and weighed hosts was 19 for WM TT, 48 for WM MP and 24 for AD EE; ‡: Estimated prevalence; *Only one host infected

Table 2

Parasitation rate for third-stage larvae (L3) *A. simplex* (s.l.) and *H. aduncum* in the musculature of the examined fish and areas.

Area	Host species	*A. simplex* (s.l.)			*H. aduncum*		
		P% (95% CI)	MI (95% CI)	A (95% CI)	P% (95% CI)	MI (95% CI)	A (95% CI)
NEA	*T. trachurus* (n = 19)	36.8 (16.3-61.6)	1.43 *	0.53 (0.17-0.96)	5.3 (0.0-26.0)	2	0.11 (0.00-0.33)
	M. poutassou (n = 44)	59.1 (43.3-73.7)	10.27 (4.38-17.32)	6.07 (2.45-10.98)	50.0 (34.6-65.4)	4.18 (2.72-6.00)	2.09 (1.20-3.19)
WM	*T. trachurus* (n = 33)	0.0 (0.0-10.6)	-	-	3.0 (0.0-15.8)	1 *	-
	M. poutassou (n = 49)	2.0 (0.0-10.9)	1 *		0.0 (0.0-7.3)	-	-
	E. encrasicolus (n = 30)	0.0 (0.0-11.6)	-	-	16.7 (5.6-34.7)	1.4 *	0.23 (0.004-0.48)
AD	*E. encrasicolus*	25.6	1.1	0.28	17.9	1.14	0.21

(n = 39)	(13.0-42.1)	(1.0-1.4)	*	(7.5-33.5)	(1-1.5)	(0.08-0.36)

NEA: North-East Atlantic, WM: West Mediterranean, AD: Adriatic, P: prevalence MI: mean intensity A: abundance, CI: Confidence interval, *Not calculable.

Table 3

Specific identification by PCR-RFLP of the ITS rDNA using *Hinf*I restriction enzyme of *Anisakis* third-stage larvae (L3) of hosts and geographical areas studied.

Area	Host species	N	n	*Anisakis simplex* (s.s.)	*Anisakis pegreffii*	Hybrid *A.s.*(s.s.)/*A.p.*	*Anisakis physeteris*
NEA	TT	9	22	10	11	1	-
	MP	10	54	39	8	1	6
WM	TT	11	32	-	28	3	1
	MP	11	19	-	13	-	6
AD	EE	3	5	-	5	-	-
Total		44		49	65	5	13

NEA: North-East Atlantic; WM: West Mediterranean; AD: Adriatic; TT: *Trachurus trachurus*; MP: *Micromesistius poutassou*; EE: *Engraulis encrasicolus*; N: number of studied hosts; n: number of larvae identified

Table 4

Measurements of the main morphological parameters of third and fourth stage larvae of *A. simplex* (s.s.) and *A. pegreffii*.

	Third-stage larvae (L3)						Fourth-stage larvae (L4) (four days cultured larvae)					
	A. simplex (s.s.) (n = 29)			*A. pegreffii* (n = 45)			*A. simplex* (s.s.) (n = 45)			*A. pegreffii* (n = 24)		
	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% Cl	Range
Body length (BL)	17.70	16.63-18.68	13.02-4.0	16.97	16.11-17.82	10.0-24.0	22.98	21.56-24.41	20.5-36.0	22.60	21.11-24.18	16.5-29.0
Body width (BW)	0.45	0.43-0.48	0.27-0.56	0.45	0.43-0.47	0.22-0.56	0.51	0.50-0.52	0.40-0.64	0.51	0.48-0.53	0.35-0.62
NR to anterior end	0.26	0.25-0.27	0.21-0.32	0.26	0.25-0.27	0.22-0.33	0.40	0.39-0.42	0.35-0.45	0.41	0.39-0.42	0.33-0.46
Ventricle length (VL)	0.92	0.88-0.96	0.71-1.15	0.87	0.82-0.91	0.57-1.15	0.94	0.89-0.99	0.81-1.25	0.86	0.80-0.91	0.59-1.13
Ventricle width (VW)	0.25	0.24-0.26	0.19-0.31	0.26	0.25-0.27	0.15-0.33	0.21	0.21-0.22	0.18-0.26	0.25	0.24-0.26	0.19-0.29
Oesophagus length (OL)	1.97	1.86-2.07	1.39-2.45	1.78	1.70-1.86	1.32-2.28	2.64	2.58-2.71	2.25-3.13	2.55	2.44-2.65	1.99-2.94
Oesophagus width (OL)	0.14	0.13-0.15	0.07-0.17	0.14	0.14-0.15	0.09-0.18	0.20	0.19-0.21	0.18-0.25	0.20	0.19-0.21	0.17-0.24
Tail length	0.09	0.08-0.10	0.05-0.13	0.09	0.08-0.09	0.05-0.12	0.14	0.13-0.14	0.10-0.19	0.13	0.12-0.14	0.09-0.19
Tail width	0.13	0.12-0.14	0.10-0.17	0.13	0.13-0.27	0.17-0.05	0.16	0.15-0.17	0.13-0.19	0.15	0.14-0.16	0.13-0.20
OL/BL (%)	11.25	10.69-11.81	8.7-15.4	10.7	10.21-11.09	7.4-13.2	11.87	11.20-12.55	8.2-17.3	11.48	10.69-12.27	9.1-15.5
VL/BL (%)	5.25	5.03-5.48	4.4-6.7	5.2	4.91-5.42	3.4-6.6	4.15	4.00-4.29	3.4-6.0	3.82	3.62-4.01	3.1-5.0
OL/VL	2.14	2.06-2.24	1.6-2.6	2.1	2.02-2.14	1.6-2.4	2.87	2.73 3.01	2.3-4.0	3.02	2.84-3.20	2.1-3.8
VL/VW	3.73	3.57-3.89	2.7-4.6	3.3	3.14-3.50	2.2-4.7	4.46	4.20-4.72	3.6-6.7	3.48	3.24-3.71	2.2-4.8

NR: nerve ring; n: number of measured larvae; CI: confidence interval; range: minim and maxim values; All measurements are given in mm.

Highlights

- *Anisakis* and *Hysterothylacium* species in consumed fish from NE Atlantic and Mediterranean were studied
- *A. simplex* (s.s.), *A. pegreffii*, *A. physeteris*, *H. aduncum* and *H. fabri* were identified.
- Geographical origin was the most influential factor in nematode abundance.
- Larval distribution in musculature was statistically analysed in Atlantic *M. poutassou*.
- Morphometric discriminant functions were generated for L3/L4 larvae of *A. simplex* (s.s.) and *A. pegreffii*.