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ORIGINAL PAPER

Occurrence of *Anisakis* and *Hysterothylacium* larvae in commercial fish from Balearic Sea (Western Mediterranean Sea)

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Abstract

This study investigates the occurrence of anisakids and raphidascarids in commercial fish from Balearic Sea (Western Mediterranean). A total of 335 fish including 19 black anglerfish (*Lophius budegassa*), 33 white anglerfish (*L. piscatorius*), 129 European hake (*Merluccius merluccius*), 30 red mullet (*Mullus barbatus*), and 124 striped mullet (*M. surmuletus*) were examined using enzymatic digestion. A total of 948 nematode larvae were isolated (prevalence 52.53%) being the highest prevalence observed in striped mullet. Forty-six larvae were identified using molecular analyses which included PCR and sequencing of the 629-bp fragment of mitochondrial cox2 gene region. *Anisakis pegreffii* (80.43%), *A. physeteris* (8.69%), *Hysterothylacium fabri* (6.52%), and *A. simplex* (4.35%) were detected based on molecular analyses of larvae. Total nematode prevalence was positively correlated with weight, length, condition factor, and maturity stage of the host and also with fishing ground depth. Statistical differences between total nematode prevalence and geographical sector of capture were observed when fishing hauls were grouped according to the abundance of sperm whales or common bottlenose dolphins. The results also corroborate that fishing water depth may play an important role in anisakid and raphidascarid parasitization.

Keywords Anisakids · Raphidascarids · *Anisakis* · *Hysterothylacium* · Teleost · Balearic Sea

Introduction

Anisakids and raphidascarids are present in a wide variety of fish and aquatic invertebrates that act as intermediate, paratenic, or definitive hosts in the life cycle of these nematodes. The accidental human ingestion of these nematodes, usually by consuming raw, undercooked or improperly processed parasitized fish or cephalopods, is associated with an important fish-borne zoonosis (Butt et al. 2004). Severe abdominal pain, digestive disorders (nausea, vomiting, and diarrhea), and/or allergic reactions are some of the reported symptoms in affected patients (del Rey Moreno et al. 2006; Umehara et al. 2007; Audicana and Kennedy 2008; Nieuwenhuizen and Lopata 2013). Earlier, anisakid infection

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has even been suggested as being a risk factor for the development of stomach or colon cancer (García-Pérez et al. 2015). A number of anisakids are known to cause human infections while raphidascarids may not be as harmful for human health as members of family Anisakidae (Shamsi 2014). Members of genera *Anisakis* and *Pseudoterranova* are the most frequently implicated in human infections, *A. simplex* and *A. pegreffii* being reported as the most common causative agent of anisakidosis (Umehara et al. 2007; Audicana and Kennedy 2008; Mattiucci et al. 2011, 2013; Mattiucci et al. 2017).

These nematodes have been described in more than 450 different species of fish and cephalopods due to their low host specificity in the larval stage (Lymbery and Cheah 2007). Anisakid and/or raphidascarid species, parasite anatomical location as well as intensity, depend not only on the host specificity but also on the seasonal and host geographical distribution. Differences in prevalence and intensity related to body size, sex, or maturity stage have been reported (Tantanasi et al. 2012; Sharif and Negm-Eldin 2013; Ferrer-Maza et al. 2014a). Nevertheless, according to Cipriani et al. (2018a), abiotic factors, such as specific oceanographic or ecological factors at the fishing area, have greater effect on infection level than biotic factors such as body size. There are no areas where it is considered that fishery products are consistently to be free of these larvae. In recent years, consumers, the fishing industry, and also health authorities are increasingly concerned about the problems caused by these parasites in human health and in the commercial value of the fishery product. Thus, epidemiological studies carried out to determine which commercial species are most parasitized and which fishing grounds have the highest rates of parasitation are of great interest (European Food Safety Authority (EFSA) 2010).

In this context, the present study examines the nematode larva prevalence in five fish species with an important commercial value and popularity in Balearic Sea waters, as well as the relation with some biotic and abiotic factors which could affect this parasitation.

Materials and methods

Sample collection

The sampling plan was carried out within the framework of the Mediterranean International Trawl Survey (MEDITS financed by DG MARE and UE members Council Regulation (EC) No. 199/ 2008) by the Spanish Institute of Oceanography between June and July 2014. Trawlings were conducted in 48 different geographical sectors (Fig. 1 and Supplementary material, Figs. 3 and 4) with a bottom trawl (model GOC-73) with a 4-m vertical opening and a 20-mm cod-end mesh size. *Depth* was *recorded* by means of a *CTD SBE-37* probe located in the mouth of the gear and ranged between 36.25 and 526 m. Further information on the sampling design and on the characteristics of the gear is available in the MEDITS Handbook (2017). A total of 335 fish specimens belonging to five different species (19 *Lophius budegassa*, 33 *L. piscatorius*, 129 *Merluccius merluccius*, 30 *Mullus barbatus*, and 124 *M. surmuletus*) were randomly collected. Each specimen was identified at species level, sexed, weighted (g), and measured at the nearest millimeter (Table 1). Maturity stage was determined according to MEDITS maturity scale (MEDITS Handbook 2017). Then, fishes were immediately frozen on board and stored at − 20 °C. Samples were transferred in frozen conditions to laboratory and stored at − 20 °C until examination.

Parasite isolation and enzymatic digestion

Once thawed, the viscera and muscle were digested separately in order to establish demographic values at the site of infection. The enzymatic digestion method used was based on the Codex Alimentarius Commission (CODEX 2004) and the EU Regulation (EC) No. 2075/ 2005. Samples were homogenized and flattened in a stomacher (Stomacher IUL Instrument, Germany) as previously reported by Llarena-Reino et al. (2013) for 90 s previous to enzymatic digestion. Digestion was carried out at 37 °C for 15 min in a fresh pepsin solution (0.1% (*w*/*v*) pepsin (2000 FIP-U/g)) and 0.063 M hydrochloric acid in distilled water at a weight/volume ratio of 1:10. Digested tissue was filtered through a sieve with a mesh size of 400 mm, flushed carefully with tap water, and then observed on a Petri dish with a stereomicroscope. Individual parasites were fixed in 70% ethanol until identification. Nematode larvae obtained from wild mackerel (*Scomber scombrus*) were flattened in the stomacher and incubated in the same conditions as controls of parasite resistance to the homogenization and enzymatic process. Condition factor (*K*) was calculated following Fulton's index as $K = W/L^3 \times 100$ (Ricker 1975), where *W* was the fish weight and *L* the fish total length.

Molecular identification

Forty-six larvae were randomly selected among the total larva samples including fish species for the molecular identification (Supplementary material, Table 6). DNA extraction was performed with the commercial kit NucleoSpin® Tissue kit (Macherey-Nagel) following the manufacturer's protocol. DNA quality and quantity were checked in a spectrophotometer NanoDrop® ND-2000 (Thermo Scientific).

The PCR reactions were carried out with 1 μl of extracted DNA (100 ng) in a final volume of 25 μl per sample, containing 2X KAPA2G Fast HotStart ReadyMix (Kapa Biosystems) with 0.4 IU of Taq polymerase (Kapa Biosystems), 0.2 mM of each dNTP, 1.5 mM of MgCl2, 0.3 μM of forward primer

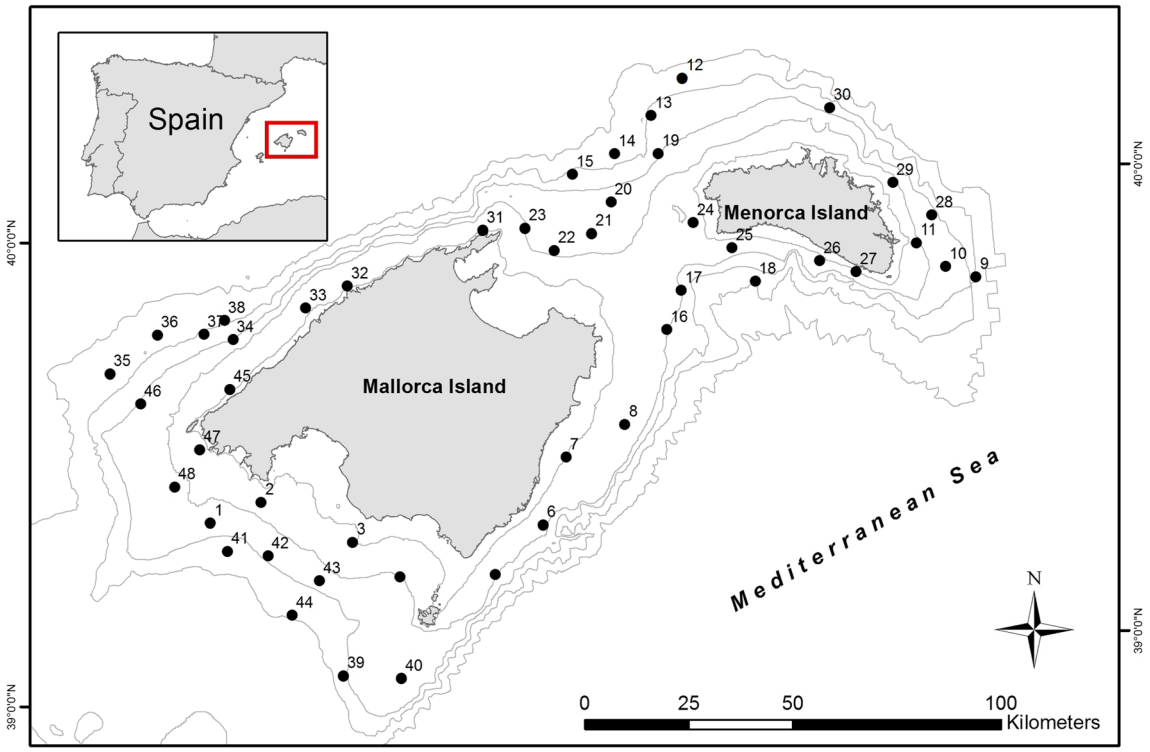


Fig. 1 Map of the studied area (West Mediterranean) showing the distribution of the sampled fishing hauls

"211F," and 0.3 μM of reverse primer "210R" for the target mitochondrial gen cox2, which allowed the sensitive amplification of 629-bp DNA fragments (Nadler and Hudspeth 2000). As positive and negative controls, 1 μl of DNA extracted from *A. simplex* or sterile water, respectively, was used. PCRs were performed in a thermal cycler (Biometra). The reactions were subjected to the following cycle conditions: 95 °C/3 min; 35X (95 °C/30 s; 55 °C/30 s; 72 °C/1 min 15 s); and 72 °C for 1 min. The completed reactions were resolved using 2% agarose gel electrophoresis and Red Safe staining in Gel Doc™ XR System (Bio-Rad). Amplicons were purified from residual reaction components using ExoProStar™ 1-Step (GE Healthcare, NJ, USA) according to the manufacturer's instructions. The nucleotide sequence determination reactions were carried out in a specialized service (Stab Vida, Portugal). The resulting chromatograms were proof-read using ChromasPro v.1.41 Technelysium Pty Ltd. Sequences were searched for identity using BLAST (Basic Local Alignment Search Tool) through web servers of the National Center for Biotechnology Information (USA).

Sequences isolated in this study ($n = 46$) and others were obtained in GenBank: *Anisakis pegreffii* (JQ900760, KU752203), *A. simplex* (JN786319, GQ338433), *A. physeteris* (KF972439, DQ116432), *Hysterothylacium fabri* (KC862609), and *H. aduncum* (KT439396, KX083579) (Valentini et al. 2006; Kijewska et al. 2009; Murphy et al. 2010; Mattiucci et al. 2013; Pekmezci et al. 2014a, b; Mateu et al. 2015; Jeon et al. 2016; Costa et al. 2018; Gaglio et al. 2018) were aligned using the ClustalW (Thompson et al. 1994) included in MEGA6 (Tamura et al. 2013). MEGA6 software also allowed inferring the best DNA substitution model for maximum likelihood (ML) tree (Tamura et al. 2013). The model with the lowest Bayesian

Table 1 Data on fish species sampled

Species	No.	Length ± SE (cm)	BW ± SE (g)	Fishing haul
Lophius budegassa	19	323.11 ± 16.3	567.37 ± 86.92	11, 22, 28, 30, 39, 40, 46, 47
L. piscatorius	33	286.4 ± 12.3	416.85 ± 45.95	6, 11, 16, 17, 21–23, 25–27, 29, 32, 33, 40, 45, 47
Merluccius merluccius	129	274.86 ± 7.2	187.3 ± 13.63	1, 4, 5, 8, 9, 12–15, 18–23, 28, 30, 34–44, 46, 47
Mullus barbatus	30	187.3 ± 6.3	84.17 ± 8.28	2, 4, 5, 22, 23, 28, 43
M. surmuletus	124	189.06 ± 2.4	80.03 ± 3.15	2, 3, 6, 7, 10, 11, 16, 17, 20, 21, 24–29, 31, 33, 34, 40–42, 45–48

No. number of specimens sampled, *SE* standard error, *BW* body weight

information criterion (BIC = 5671.974) score was Hasegawa-Kishino-Yano with gamma distribution and invariant sites (HKN+G+I). The tree was computed in MEGA6 with 1.000 bootstrap replications.

Statistical analysis

Quantitative parasite descriptors such as prevalence (P), mean intensity (MI), and mean abundance (MA) were calculated according to Bush et al. (1997). Normality of the data was tested exploring the data and using a Kolmogorov–Smirnov and Shapiro test. Pearson's correlation coefficient (r) was applied to determinate the degree of association between prevalence and biological (weight, length, body condition) or environmental (depth) variables. Associations between total nematode prevalence and the non-parametric variables (sex, maturity, and geographical sector of capture) were explored using the correlation coefficient (r) with the Kruskal–Wallis test. For the geographical sector of capture, the fishing hauls were stratified into five zones (Balearic-Sub-Basin, Algerian-Sub-Basin, Mallorca Channel, Menorca Channel, and North Balearic Basin) based on the differences in seabed topography and water currents according to Acosta et al. (2004) (Supplementary material). According to this stratification, associations between total prevalence as well as different nematode species prevalences and geographical sector of capture were calculated. The fishing hauls were also stratified into two zones based on the abundance of sperm whales (*Physeter macrocephalus*) (Rendell et al. 2014) or on the abundance of common bottlenose dolphins (*Tursiops truncatus*) (Gonzalvo et al. 2008) (Supplementary material). According to these classifications, associations between nematode prevalences and geographical sector of capture were calculated using a non-parametric ANOVA test (Kruskal–Wallis). Statistical significance was established at $p < 0.01$ or $p < 0.05$. All statistical analyses were performed using SPSS statistics version 23.0.

Results

Anisakid and raphidascarid larva prevalences observed in the five fish species ranged between 25.58% in *Merluccius merluccius* and 78.29% in *Mullus surmuletus*. A total of 948 larvae were isolated from the viscera (80.7%) and muscle (19.3%). Table 2 shows P, MI, and MA of total nematodes either in the muscle or in the viscera of sampled fish.

Forty-six sequences obtained in this study shared 100% nucleotide identity with sequences of *A. pegreffii* ($N = 37$), *A. physeteris* ($N = 4$), *A. simplex* ($N = 2$), and *H. fabri* (N = 3) deposited in the GenBank mtDNA cox2 tree, constructed with a total of 510 sites and 55 sequences, which showed that 3 nematode sequences were clustered with *H. fabri* with bootstrap value of 100% and 43 sequences were grouped in the *Anisakis* clade (Fig. 2). Within this clade, 4 sequences were clustered in the subclade of *A. physeteris* with bootstrap value of 100%, 2 sequences were included in the subclade of *A. simplex* with very high value of bootstrap (99%), and 37 sequences in the subclade of *A. pegreffii* with bootstrap values of 97%.

A significant correlation (Table 3 and Supplementary material, Table 5) was found between total prevalence and weight (p value 0.0001), length (p value 0.0001), condition (p value 0.0001), host maturity stage (p value 0.0001), and depth (p value 0.0001). A significant correlation was found between total prevalence and host sex (p value 0.044) being the prevalence lower in fish with undetermined sex than in males or females. No significant differences in prevalence were seen between females and males. Also, a significant correlation was observed between *Anisakis* spp. and *A. pegreffii* prevalence and weight and length as well as between *Anisakis* spp. prevalence and condition host maturity stage and depth. When fishing hauls were grouped based on the differences in seabed topography and water currents, no significant correlation between *Anisakis* spp., *A. pegreffii*, or overall nematode prevalence and geographical sector of capture was observed (Table 3). Nevertheless, statistical differences between total prevalences and geographical sector of capture were observed when fishing hauls were grouped according to the abundance of sperm whales ($\chi^2 = 9.143$, $p = 0.02$) or common bottlenose dolphins ($\chi^2 = 14.59$, $p = 0.0001$).

Discussion

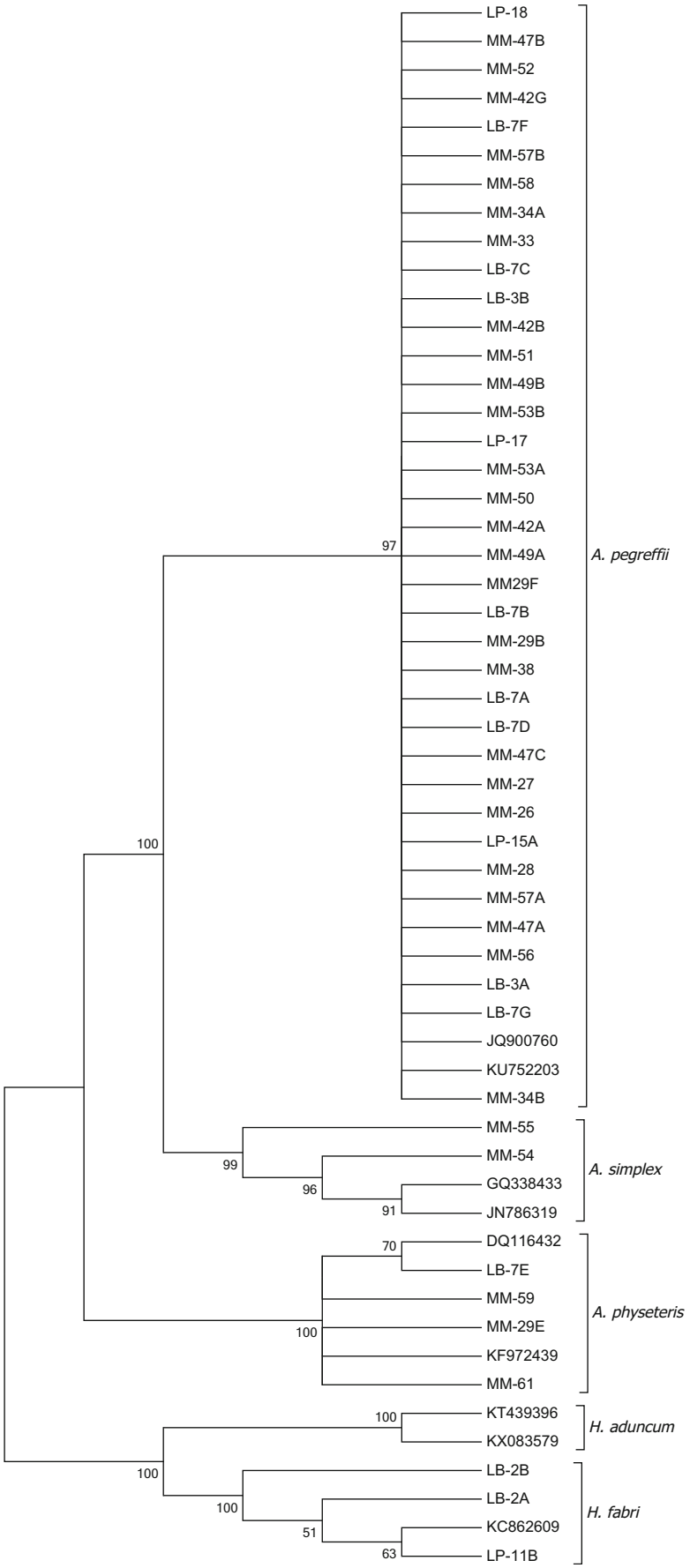
In this study, conducted in fish from Balearic Sea waters, the highest nematode prevalence was detected in the striped mullet (*M. surmuletus*) (78.29%). Other studies have reported a lower prevalence in this fish species sampled from other Mediterranean waters, 69% from Algerian Sea (Hassani et al. 2015), 58.9% from Tyrrhenian Sea (Arguleo et al. 1997), 37.5% from Sardinia waters (Angelucci et al. 2011), 36.7% from Libyan waters (Kassem and Bowashi 2015), and 33.3% from the Mediterranean Spanish coast (Pulleiro-Potel et al. 2015). Similarly to our study, Ferrer-Maza et al. (2014a) reported a high prevalence (60%) in viscera of *M. barbatus* from the eastern coast of Spain, with a similar mean intensity (3.95 ± 5.94 parasites). The most prevalent species reported by those authors was *Hysterothylacium fabri* (63.9%). On the other hand, lower prevalences were detected in red mullet from Ligurian Sea (Serracca et al. 2013) (25.6%), from the Valencian coast (Mediterranean Spanish coast) (41%) (Debenedetti et al. 2013), and from Turkish Mediterranean coast (41.6%) (Pekmezci et al. (2014a, 2014b).

The monkfish *Lophius piscatorius* showed a total anisakid and raphidascarid larva prevalence of 42.42%, with the larvae

Table 2 Prevalence, mean intensity, and mean abundance of anisakid and raphidascarid larvae in sampled fish

Species	Prevalence (%) CI[a]			Mean intensity (range)			Mean abundance (CI)		
	Total	Muscle	Viscera	Total	Muscle	Viscera	Total	Muscle	Viscera
Lophius budegassa	36.84 (0.152–0.585)	10.53 (− 0.033–0.243)	36.84 (− 0.152–0.585)	3.43 (1–7)	1 (1)	3.14 (1–7)	24/19	1.26 (0.236–2.290)	2/19
L. piscatorius	42.42 (0.256–0.593)	6.06 (− 0.021–0.142)	39.39 (0.227–0.561)	3.93 (1–12)	1.50 (1–2)	4 (1–12)	55/33	1.67 (0.634–2.699)	3/33
Merluccius merluccius	25.58 (0.181–0.331)	6.98 (0.026–0.114)	20.93 (0.139–0.280)	1.82 (1–8)	1.44 (1–3)	1.74 (1–8)	60/129	0.46 (0.268, − 0.662)	13/129
Mullus barbatus	70 (0.536–0.864)	26.67 (0.108–0.425)	60 (0.425–0.775)	9.71 (1–41)	21 (1–39)	4.71 (1–16)	204/30	6.8 (2.941–10.659)	105/30
M. surmuletus	78.29 (0.712–0.854)	27.42 (0.196–0.353)	75 (0.692–0.841)	5.99 (1–68)	0.59 (1–6)	5.4 (1–67)	605/124	4.88 (3.466–6.293)	60/124
Total	52.53 (0.472–0.579)	16.12 (0.244–0.381)	47.16 (0.867–0.952)	5.39 (1–68)	1.04 (1–39)	4.35 (1–67)	948/335	2.83 (2.154–3.505)	183/335

[a] CI = Confidence interval


LP-18
MM-47B
MM-52
MM-42G
LB-7F
MM-57B
MM-58
MM-34A
MM-33
LB-7C
LB-3B
MM-42B
MM-51
MM-49B
MM-53B
LP-17
MM-53A
MM-50
MM-42A
97
MM-49A
MM29F
LB-7B
MM-29B
MM-38
LB-7A
LB-7D
MM-47C
MM-27
MM-26
100
LP-15A
MM-28
MM-57A
MM-47A
MM-56
LB-3A
LB-7G
JQ900760
KU752203
MM-34B
A. pegreffii
MM-55
MM-54
99
GQ338433
96
JN786319
91
A. simplex
70
DQ116432
LB-7E
MM-59
MM-29E
100
KF972439
MM-61
A. physeteris
100
KT439396
KX083579
H. aduncum
100
LB-2B
100
LB-2A
KC862609
51
LP-11B
63
H. fabri

◀ **Fig. 2** Maximum likelihood analysis showing the taxonomic position of the nematode larvae infecting fishes in the Mediterranean Sea. Numbers at branch nodes indicate bootstrap confidence values in percent. MM, *Merluccius merluccius*; LP, *Lophius piscatorius*; LB, *Lophius budegassa*

molecularly identified as *A. pegreffii* or *Hysterothylacium fabri*. Unfortunately, data on the presence of nematode larvae in this species from the Mediterranean Sea are very scarce. Farjallah et al. (2008) sampled two white anglerfish from North African coasts of the Mediterranean Sea, reporting an infection by *A. pegreffii*, while Akmirza (2013) reported the presence of *Contracaecum aduncum* in one specimen fished in Turkish waters. Regarding black anglerfish, Pulleiro-Potel et al. (2015) reported a prevalence of 5.56% from the Spanish east coast, with the isolated larvae belonging to the *Anisakis* or *Hysterothylacium* genera; in this study, a much higher prevalence of anisakid and raphidascarid larvae (36.84%) was detected in black anglerfish. Anisakid definitive host abundance could explain the higher prevalence detected in our study compared to other studies conducted in the same fish species from other Mediterranean areas.

The Balearic Sea is recognized for its high cetacean richness (Rendell et al. 2014; Arcangeli et al. 2017), and high density of parasites correlates with high abundance of marine mammals, which are the definitive hosts of several anisakid nematodes (Rello et al. 2009). However, in our study, only a moderate nematode prevalence was detected in European hake. This corroborates a recent study which described remarkably high levels of infection in hakes from the Adriatic/Ionian Sea compared to infection rates in similar length hakes from the Western Mediterranean (Cipriani et al. 2018b).

Our study indicates that *A. pegreffii* exhibited the highest prevalence values. This is the most widespread anisakid species affecting commercial fish from Mediterranean waters (Levsen et al. 2018), and it has an important zoonotic potential. In fact, it has been demonstrated that *A. pegreffii* is able to cause gastric (Fumarola et al. 2009; Mattiucci et al. 2013), intestinal (Mattiucci et al. 2011; Mladineo et al. 2016), and gastroallergic anisakiasis (Mattiucci et al. 2013). Mattiucci et al. (2004) identified *A. pegreffii* in hakes from the Balearic Sea (prevalence 14.3%) as well as in all the hakes sampled from other areas of the Mediterranean Sea, except in the Levantine Sea (off the Cypriot coast). Nevertheless, according to these authors, *A. physeteris* was the most prevalent species in hakes from the Balearic Islands (prevalence 85.7%). Contrastingly, our study shows that 83.87% of the molecularly identified larvae from hakes were *A. pegreffii*, 9.68% were *A. physeteris*, and 6.45% were *A. simplex*. Mattiucci et al. (2004) sampled European hake juveniles (the majority < 35 cm total length) and adults (the majority > 35 cm), meaning the fish host size (much bigger in our study (274.86 ± 7.2)) could explain these differences. As it has been previously suggested, fish size is a confounding variable in parasite studies, especially in long-lived species such as *Anisakis* larvae (Mattiucci et al. 2015).

Additionally, a positive correlation between length, weight, and corporal condition of the host and total nematode prevalence was observed in our study. This can be attributed to the cumulative parasitation over time through the diet (Adroher et al. 1996; Mattiucci et al. 2004; Rello et al. 2009) and to the change in diet (Valero et al. 2006; Levsen et al. 2018). Indeed, a prediction model for the number of existing anisakids in the fish by measuring only its length has even been proposed in *Trachurus trachurus* (Tantanasi et al. 2012). Furthermore, a recent study considers fish host body size as a major predictor of *Anisakis* sp. occurrence in most fish species (Levsen et al. 2018). However, a few exceptions to this trend have been described in some species such as mackerel (Levsen et al. 2018) and European anchovy (Rello et al. 2009; De Liberato et al. 2013; Ferrer-Maza et al. 2016).

With regard to the reproduction variable, a positive correlation between the host maturity stage and total nematode prevalence was observed. Prevalence was higher in specimens during the spawning capable phase than those during the

Table 3 Correlation coefficients between anisakid and raphidascarid prevalence and biological and environmental variables in sampled fish from Balearic Sea

Species	Weight, r (p)	Length, r (p)	K, r (p)	Sex, r (p)	Maturity, r (p)	Depth, r (p)	Sector, r (p)
Total isolated nematodes	$r^2 = 0.263$ (0.0001)**	$r^2 = -0.323$ (0.0001)**	$r^2 = 0.262$ ($p = 0.0001$)**	$X^2 = 6.237$ (0.044)*	$\chi^2 = 78.75$ (0.0001)**	$r^2 = -0.223$ (0.0001)**	$\chi^2 = 8.544$ (0.074)
Total molecularly identified anisakids							
Anisakis spp.***	$r^2 = 0.249$ (0.0001)**	$r^2 = 0.270$ (0.0001)**	$r^2 = 0.134$ (0.0001)**	$\chi^2 = 5.56$ (0.135)	$\chi^2 = 16.608$ (0.034)*	$r^2 = 0.284$ (0.0001)**	$\chi^2 = 8.745$ (0.068)
A. pegreffii	$r^2 = 0.616$ (0.005)**	$r^2 = 0.566$ (0.011)*	$r^2 = 0.213$ (0.382)	$\chi^2 = 5.296$ (0.071)	$\chi^2 = 3.299$ (0.770)	$r^2 = 0.209$ (0.391)	$\chi^2 = 8.815$ (0.066)

r correlation coefficient, K body condition score, p p value

*$p < 0.05$

**$p < 0.01$

***_Anisakis_ spp. integrate the three species of the genus _Anisakis_ detected in the study

regenerating phase. This might indicate that they continue to feed during reproductive stage (since fish get infected while feeding). This assumption, previously suggested for *M. barbatus* (Ferrer-Maza et al. 2014a), is supported by the fact that some of these fish species in the Mediterranean Sea behave like income breeders, i.e., the energy assigned to reproduction comes from synchronous feeding. Thus, nourishing continues throughout the spawning period (Ferrer-Maza et al. 2014a, b), increasing the risk of parasitized intermediate host intake. Another explanation could be the potentially limited investment in immunity during the breeding; this investment in reproduction at the expense of immunosuppression has been previously suggested. Skarstein et al. (2001) reported that spawning salmonid Arctic charr (*Salvelinus alpinus*) were more susceptible to parasite infection than resting ones.

A significant correlation was observed between total nematode prevalence and geographical sector of capture when fishing hauls were grouped based on common bottlenose dolphins or sperm whale abundance. Dolphins and sperm whales are considered the main definitive hosts of *A. pegreffii* and *A. physeteris* respectively (Klimpel and Palm 2011). The inshore waters of the Balearic Islands, where the fishing hauls of the present study were located, provide a critical habitat for dolphins. However, according to Gonzalvo et al. (2008), common bottlenose dolphins are more abundant in northeast and southwest of the Balearic Archipelago, while the south and east present a higher abundance of sperm whales (Rello et al. 2014). The uneven distribution of these potential final hosts would explain the observed correlation between total nematode prevalence and geographical sector of capture observed in our investigation.

In regard to depth and nematode prevalence, a positive correlation was detected in red mullet (p value 0.002).This correlation cannot be attributed to the presence of larger red mullet inhabiting deeper zones. A Kruskal–Wallis test was conducted to explore the association between red mullet length or weight and depth, and no significant correlation was observed (p value 0.61 and 0.84 respectively). A positive correlation was also observed between depth and total nematode prevalence. These results corroborate that fishing water depth appears to be an important risk factor for anisakid and raphidascarid parasitization of some commercially important fish. This has been previously reported in sardines (*Sardina pilchardus*), anchovies (*Engraulis encrasicolus*), and greater forkbeard (*Phycis blennoides*) (Pulleiro-Potel et al. 2015). In fact, specific oceanographic or ecological factors have been suggested to have a wider impact on the anisakid infection level than specific fish host characteristics such as body size (Cipriani et al. 2018a). Taking into account the habitat use of marine cetaceans, final hosts of some anisakid genera, our results suggest that fishing boats should fish in shallow waters in order to reduce risk of anisakid infection.

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