



Molecular epidemiology and risk factors for *Anisakis simplex* s.l. infection in blue whiting (*Micromesistius poutassou*) in a confluence zone of the Atlantic and Mediterranean: Differences between *A. simplex* s.s. and *A. pegreffii*.

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ABSTRACT

Our study determined parameters of parasitization of *Anisakis simplex* s.l. in *Micromesistius poutassou* in a confluence zone of the Atlantic and Mediterranean, with a total prevalence of 82%. Also, in the three seasons analyzed, high prevalence's values were found, reaching 100% in spring; however mean intensity and abundance values were higher in winter. The use of molecular techniques to differentiate between *Anisakis* genotypes of the larvae characterized allowed obtaining values of 99.7% *Anisakis simplex* s.l. (50.1% *A. simplex* s.s., 42.9% *A. pegreffii*, 7.0% *A. simplex* s.s. - *A. pegreffii* hybrids) and 0.3% *A. typica*. The infections found in the fish were of both single and mixed species, in all the different possible combinations. The presence of *A. simplex* s.l. in the viscera varied according to genotype and season. Likewise, factors associated with the presence of the parasite in the ventral or dorsal musculature were different, where *A. simplex* s.s. proportion was double than that of *A. pegreffii*. The ecology of the two sibling species with regard to their location in fish and the influence of the season were different.

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1. Introduction

Anisakis simplex s.s. is the most abundant species of the family Anisakidae in the Atlantic Ocean, whilst *Anisakis pegreffii* is predominant in the southern hemisphere and the Mediterranean Sea (Ceballos-Mendiola et al., 2010; Martín-Sánchez et al., 2005; Mattiucci and Nascetti, 2008; Piras et al., 2014). Pelagic, mesopelagic and demersal fish act as intermediate hosts for both species (Olivares, 2012). The waters surrounding the Iberian Peninsula are known to be a zone of confluence where the two species live in sympatric conditions, in proportions which vary according to the different coastal areas (Hermida et al., 2012; Martín-Sánchez et al., 2005). These nematode larvae were classified in type I, that have the esophageal ventricle linked to the intestine with an oblique form, and type II (Berland, 1961), and reach their adult stage inside marine mammals. Humans can be accidentally infected by the larva in its third stage (L3) through consuming parasitized fish and consequently suffer digestive problems of varying severity (epigastric pain, gastrointestinal ulcer, diarrhoea, vomit or granuloma,

among others), allergic reactions due to contact with and/or exposure to the allergens of the parasite, or a combination of the two (Castán et al., 2002; Daschner et al., 2000; Matsuoka et al., 1994; Tadanori et al., 1992). Anisakiasis has been seen as an emerging health problem since the 1990s, when many wrongly diagnosed clinical cases were revised (Audicana and Kennedy, 2008). As the musculature of the fish is the part which is usually consumed, the migration of the *Anisakis* larvae to this part constitutes the main source of infection. Differences in the pathogenicity of *A. simplex* s.s. and *A. pegreffii* have been reported (Romero et al., 2013). In addition to the significant risks it poses to public health, the presence of *Anisakis* spp. larvae in fish also affects the quality of the product, with its ensuing economic consequences. Blue whiting (*Micromesistius poutassou*) is a bathypelagic species of the gadid family which can live at a depth of 150 m to 3000 m, but most frequently at 200 to 400 m. It feeds mainly on small crustaceans, although larger individuals also prey on small fish and cephalopods (Cohen et al., 1990; Svetovidov, 1986). The blue whiting is common in the northeast Atlantic Ocean and the Mediterranean Sea and generally registers high rates of parasitization by these nematodes (Chía et al., 2010; Cruz et al., 2007; De la Torre Molina et al., 2000; Fernández et al., 2005; Piras et al., 2014; Viu et al., 1996) and differences in the prevalence of *Anisakis* spp. infection exist between the Atlantic and Mediterranean coasts of the Iberian Peninsula (Madrid et al., 2012; Ruiz-Valero et al., 1992). This host species is highly relevant in commercial and fishing terms, with high annual catch quotas. According to the Food and Agriculture

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Organization of the United Nations, blue whiting was the fifth most important capture fish species in 2006 (<http://www.fao.org/fishery/sofia/en>). This fact, together with its low market price, makes it a commonly consumed fish in southern Europe. The purpose of this research is to study the molecular epidemiology and risk factors for the infection of this fish in a confluence zone of the Atlantic Ocean and Mediterranean Sea, to obtain values for the various indicators of parasitism and ecological factors by these two species of the *A. simplex* s.l. complex and to investigate possible differences between them.

2. Materials and methods

2.1. Fishing area

The Gulf of Cadiz is an inlet of the Atlantic Ocean on the southwestern coast of the Iberian Peninsula (36°49'60"N, 71°10'00"W) and its waters converge with the Mediterranean Sea (Fig. 1). The fish were collected through seine fishing and purchased at a market of the city Sanlúcar de Barrameda; the origin and collection method were verified at the point of sale by the identifier that accompanies the product and ensures traceability from capture.

2.2. Host and parasites

Third stage *Anisakis* larvae (L3) were obtained from blue whiting (*M. poutassou*) caught in Gulf of Cadiz waters in autumn, spring and winter. They were opened longitudinally along the abdomen to extract the free larvae in the body cavity and the viscera were placed in 0.9% physiological saline solution in order to isolate the parasite. These were then put into a pepsin-HCl solution (pH 2) to simulate digestive conditions (modified after McGladdery, 1986) at 36 °C for 2 h. The body cavity of the fish was rinsed to make sure that we had obtained all the larvae. The ventral and dorsal musculature were then removed and subjected to digestion, at 36 °C with periodic shaking for 3 h. The result of each digestion was observed through a stereoscopic microscope and the larvae were counted.

2.3. Morphological identification of the parasites

The parasites were observed *in vivo* under a stereoscopic microscope to search for the main characteristics of *Anisakis* (Berland, 1961; Petter and Maillard, 1988). Upon their identification as *Anisakis* type I or II larvae, they were preserved at –20 °C to await identification through molecular techniques.



Fig. 1. Map of the fish capture region, the Gulf of Cádiz. This photo is licensed under a Creative Commons Attribution-NonCommercial-Share Alike 4.0 license.

2.4. Genetic identification of the parasites

We extracted genomic DNA from the larvae with the RealPure kit (Ref RBMEG01) according to the manufacturer's instructions. We amplified the rDNA fragment ITS1–5.8S–ITS2 using the primers NC5 (Forward), 5'-GTAGGTGAACCTGCGGAAGGATCATT-3', and NC2 (Reverse), 5'-TTAGTTTCTTTTCCTCCGCT-3', (Zhu et al., 1998). The PCR conditions were: first cycle of 94 °C for 5 min, 60 °C for 30 s, 72 °C for 90 s; 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s; and a final extension of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 5 min and then kept at 4 °C until tubes were removed (Zhu et al., 2000). Restriction Fragment Length Polymorphism (RFLP) is performed through the digestion of the PCR amplified fragment with at least one restriction enzyme; in this case it was performed using the restriction enzymes *Hinf I* and *Taq I* at 0.5 U/μL (Fastdigest, Thermo Scientific, Germany), used individually. The results were visualized through electrophoresis in 3% agarose gel, which permitted the sibling species of the *A. simplex* complex to be identified according to the band pattern (D'Amelio et al., 2000; Kijewska et al., 2000; Martín-Sánchez et al., 2005; Pontes et al., 2005; Umehara et al., 2006). In the *A. simplex* s.s. controls, digestion with the *Hinf I* enzyme produced two fragments of 620 and 250 bp and a third weaker one of 100 bp; the *Taq I* endonuclease provided three fragments: one of 430 bp, one of 400 bp and a weaker one of 100 bp. The *A. pegreffii* controls presented a pattern of three bands of 370, 300 and 250 bp for the *Hinf I* enzyme and three of 400, 320 and 150 bp for the *Taq I* enzyme. For hybrid individuals, the PCR-RFLP band pattern with the two restriction enzymes, *Hinf I* and *Taq I*, is the sum of the patterns generated for *A. simplex* s.s. and *A. pegreffii* (Fig. 2).

2.5. Quantitative epidemiology and risk factors analysis

The following epidemiological parameters were calculated: prevalence (P), mean intensity (I) and mean abundance (A) of infection as defined by Bush et al. (1997).

$$P = \frac{\text{No of parasite hosts}}{\text{No of analyzed hosts}} \times 100 (\%)$$

$$I = \frac{\text{No of parasites}}{\text{No of infected hosts}}$$

$$A = \frac{\text{No of parasites}}{\text{No of analyzed hosts}}$$

Risk factors for *Anisakis* infection were identified through univariate and multivariate logistic regression analysis. The association of the

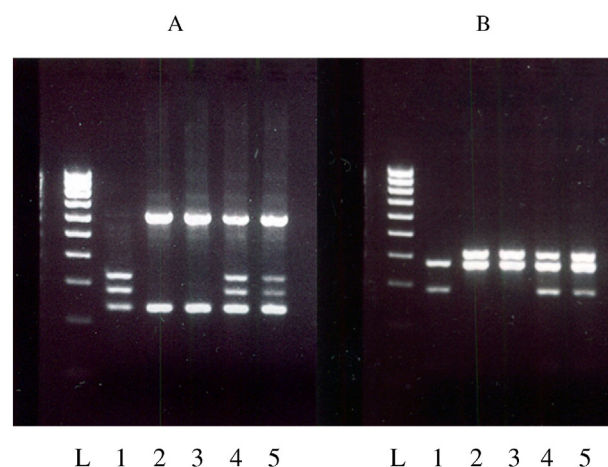


Fig. 2. RFLP profiles obtained by digestion of ITS region with the restriction enzymes *Hinf I* (A) and *Taq I* (B). Lanes: 1 *Anisakis pegreffii*; 2 *Anisakis simplex* s.s. control; 3 *Anisakis simplex* s.s.; 4 *Anisakis simplex* s.s. - *Anisakis pegreffii* hybrid genotype control; 5 *Anisakis simplex* s.s. - *Anisakis pegreffii* hybrid genotype; L 1000 bp DNA ladder.

following dependent variables was investigated: a. total parasitization, b. viscera parasitization, c. ventral musculature parasitization, d. dorsal musculature parasitization, e. parasitization ratio of *A. pegreffii* to *A. simplex* s.s. Different parameters related to the fish (weight, length), their capture (season), and the parasite (location, genotype, total number of larvae) were used as independent variables. IBM SPSS Statistics 20.0. was used for the data analysis.

3. Results

3.1. Host

A total of 100 blue whiting were analyzed measuring 17.50 to 28.50 cm long (mean: 22.7 cm $\pm$ 1.9) and weighing 29.70 to 110.50 g (mean: 73.8 g $\pm$ 17.5). The two variables were strongly correlated: Pearson correlation = 0.885, $p < 0.001$; correlation between weight and squared length is slightly lower (Pearson correlation = 0.875, $p < 0.001$). 63% (63/100) of the fish were caught in autumn, 17% (17/100) in winter and the remaining 20% (20/100) in spring.

3.2. Epidemiological parameters

Of the 100 blue whiting analyzed, 82% (82/100) were parasitized. Table 1 shows the parasitization values (prevalence, intensity and abundance) both in total and according to season, indicating the location of the parasite in the host. Fig. 3 shows the variation in infection parameters according to the weight and length of the fish.

The total number of *Anisakis* L3 larvae collected was 1350. These were all morphologically identified as L3 Type I. Only three fish were found with more than 150 parasites: 171, 229 and 328 larvae; in the rest the number varied from 0 to 78 parasites (mean: 13.5 $\pm$ 43.5). Table 1 indicates the number of larvae collected in the different locations inside the host and according to season.

3.3. Genetic identification of *Anisakis* larvae by PCR-RFLP

A number of larvae, selected at random from each fish and each location, were identified by molecular techniques: in total, 400 of the 1350 larvae collected (29.6%). In view of its relevance in terms of human infection, a high proportion (47.7%) of the larvae taken from the musculature were identified (155/325). The overall distribution was as follows: 11% of larvae found free in the abdominal cavity (45/

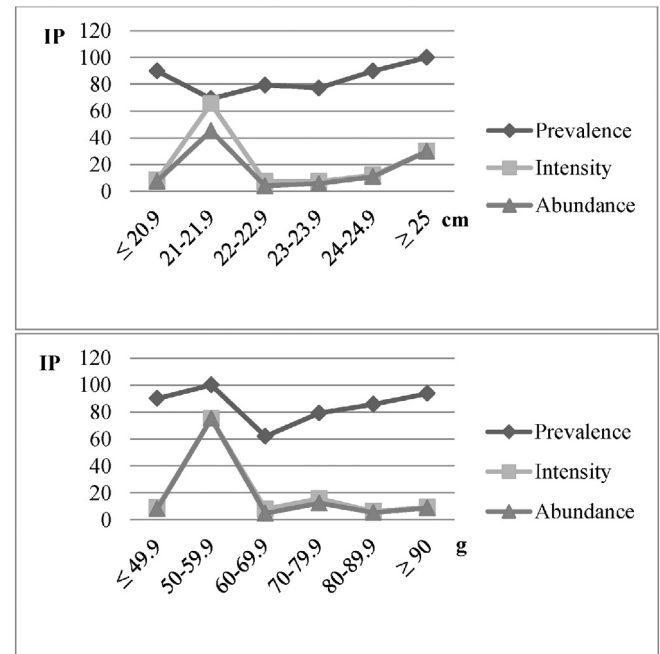


Fig. 3. Infection parameters (IP) according to weight (g) and length (cm) of host.

400), 50.0% of larvae encysted in the viscera (200/400), 26.5% of larvae found in the ventral musculature (106/400) and 12% in the dorsal musculature (49/400). By season, 25% of *Anisakis* type I L3 larvae were analyzed in autumn (99/400), 26.8% in winter (107/400) and 48.5% in spring (194/400).

The distribution of the identified *Anisakis* species was as follows: 99.7% (399/400) of the larvae belonged to the *A. simplex* s.l. complex, and only one of the 400 larvae (0.3%) was identified as a different species, *A. typica*. Within the *A. simplex* s.l. complex, 50.1% (200/399) of the larvae belonged to *A. simplex* s.s., 42.9% (171/399) to *A. pegreffii* and the remaining 7.0% (28/399) were hybrid genotypes of the two former species (Fig. 2). The proportion of larvae belonging to the different genotypes of the *A. simplex* s.l. complex according to their different locations within the host (cavity, viscera, ventral musculature and dorsal musculature) is shown in Fig. 4, and the identification of the genotypes by season, in Fig. 5. The proportion of *A. simplex* s.l. species according to the length of the host is described in Table 2.

The parasitized fish had both infections with a single *Anisakis* species and mixed infections in all the possible combinations. 6% (6/100) of the fish were parasitized only by *A. simplex* s.s., 33% (33/100) by *A. pegreffii*,

Table 1
Total and seasonal parasitization values according to location within the host.

	Parameter of infection	Total	Corporal cavity	Viscera	Ventral Musculature	Dorsal Musculature
Total	P	82% (82/100)	32% (32/100)	73% (73/100)	37% (37/100)	18% (18/100)
	I	16	0.65	13	7.4	2.9
	A	13.5	0.54	9.71	2.72	0.53
	N	1350	54	971	272	53
Autumn	P	75% (47/63)	27% (17/63)	63% (40/63)	14% (9/63)	5% (3/63)
	I	2.7	1.2	2.3	1	1
	A	2.0	0.32	1.5	0.19	0.1
	N	128	20	93	12	3
Winter	P	88% (15/17)	35% (6/17)	76% (13/17)	65% (11/17)	35% (6/17)
	I	46	2	47	3.7	3
	A	40	0.5	36	2.4	1.2
	N	682	9	612	41	20
Spring	P	100% (20/20)	50% (10/20)	100% (20/20)	85% (17/20)	45% (9/20)
	I	27	2.5	13	13	3
	A	27	1.3	13	11	1.5
	N	540	25	266	219	30

P = prevalence, I = mean intensity; A = mean abundance, N = number of larvae.

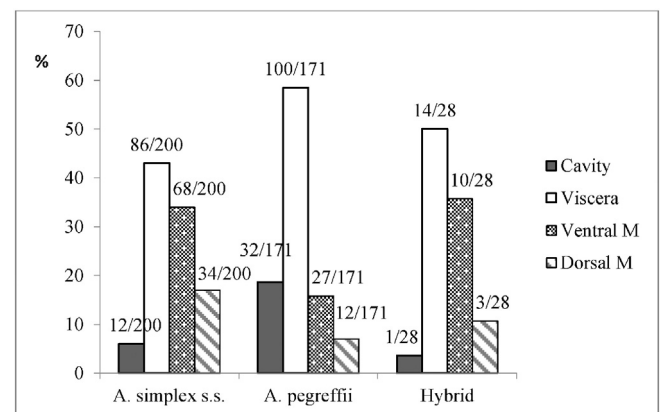


Fig. 4. Percentage (%) of genotypes of the *A. simplex* s.l. larvae found in different locations within the host. Ventral M = Ventral musculature, Dorsal M = Dorsal musculature.

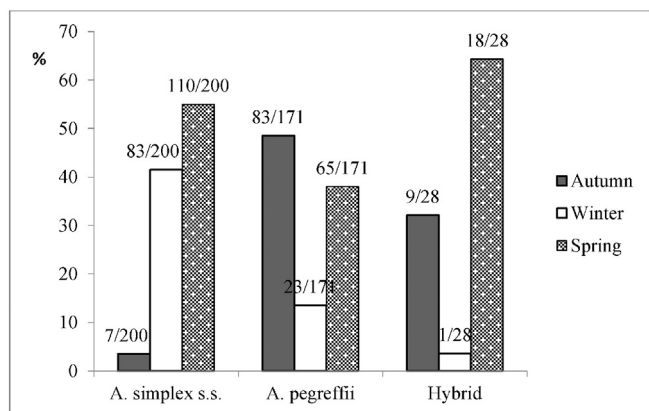


Fig. 5. Seasonal percentage (%) of genotypes of the *A. simplex* s.l. larvae identified.

4% (4/100) by the hybrid genotype, 19% (19/100) by *A. simplex* s.s. and *A. pegreffii*, 10% (10/100) by *A. simplex* s.s., *A. pegreffii* and the hybrid, 2% (2/100) by *A. simplex* s.s. and the hybrid, 7% (7/100) by *A. pegreffii* and the hybrid, and 1% (1/100) by *A. simplex* s.s., *A. pegreffii*, the hybrid and *A. typica* (Table 3). The prevalence of infection in blue whiting by the different genotypes of *Anisakis* spp. in the different locations within the fish is shown in Table 3.

3.4. Analysis of risk factors

3.4.1. Factors associated with general parasitization

The weight of the blue whiting is not associated with the general parasitization of the fish by *A. simplex* s.l. ($p = 0.61$), nor is its length ($p = 0.47$). The capture season is also not shown to be associated ($p = 0.51$). The rest of the variables could not be taken into consideration.

3.4.2. Factors associated with the presence of the larva in the viscera

100% of the larvae found in the viscera were encysted. With regard to the other independent variables investigated, the univariate analysis showed association with the length of the fish ($p = 0.045$), the genotype ($p = 0.010$) and the season ($p < 0.001$).

In the construction of the multivariate model, the possible existence of interactions between the different variables was taken into account. In the final model the length variable loses its significance, so the variables that remain are “genotype” ($p = 0.004$: ref. *A. simplex* s.s.: *A. pegreffii*, OR = 2.9, $p < 0.001$; hybrid, $p = 0.47$) and “season” ($p < 0.001$: ref. spring; autumn $p = 0.26$; winter OR = 3.3, $p < 0.001$), with interaction between the two ($p = 0.005$: autumn $\times$ *A. pegreffii*, $p = 0.64$; autumn $\times$ hybrids, $p = 0.47$; winter $\times$ *A. pegreffii*, $p = 0.001$, OR = 0.15; winter $\times$ hybrids, $p = 1.0$).

3.4.3. Factors associated with the presence of the larva in the ventral musculature

The univariate analysis shows association with the genotype ($p < 0.001$) and the season ($p < 0.001$). The weight of the fish was close to significance ($p = 0.10$), so it was taken into account in the

Table 2
Percentage (%) of genotypes of *A. simplex* s.l. according to length (cm) of host.

Length (cm)	N° L3	As (%)	Ap (%)	H (%)
≤20.9	38	4.3 (17/400)	5.3 (21/400)	–
21–21.9	76	15 (58/400)	3.8 (15/400)	0.8 (3/400)
22–22.9	74	5.5 (22/400)	11 (42/400)	2.5 (10/400)
23–23.9	58	6.5 (26/400)	7.3 (29/400)	0.8 (3/400)
24–24.9	48	2.5 (10/400)	8.0 (32/400)	2 (6/400)
≥25	106	17 (67/400)	8.0 (32/400)	2 (6/400)

As = *A. simplex* s.s.; Ap = *A. pegreffii*; H = hybrid *A. simplex* s.s.–*A. pegreffii*.

Table 3

Prevalence (%) of infection in blue whiting by the different genotypes of *Anisakis* spp. in the different locations within the fish. These percentages indicate the number of fish parasitized by the different genotypes of *Anisakis* spp. respect to the total number of blue whiting analyzed (100).

	Cavity	Viscera	Ventral M	Dorsal M	Total
As	6	8	12	5	6
Ap	23	43	8	5	33
As + Ap	2	9	9	4	19
H	1	3	3	1	4
As + Ap + H	–	1	1	1	10
As + H	–	2	2	1	2
Ap + H	–	7	1	–	7
As + Ap + At + H	–	–	1	–	1
Total	32	73	37	17	82

Ventral M = Ventral musculature, Dorsal M = Dorsal musculature; As = *A. simplex* s.s.; Ap = *A. pegreffii*; H = hybrid; At = *A. typica*; As + Ap = mixed infection by *A. simplex* s.s. and *A. pegreffii*; As + Ap + H = mixed infection by *A. simplex* s.s., *A. pegreffii* and hybrid; As + H = mixed infection by *A. simplex* s.s. and hybrid; Ap + H = mixed infection by *A. pegreffii* and hybrid; As + Ap + At + H = mixed infection by *A. simplex* s.s., *A. pegreffii*, *A. typica* and hybrid.

construction of the multivariate model, as was the possible existence of interaction between the variables “genotype” and “season”.

The final multivariate model consists of the variables “genotype” ($p = 0.20$: ref. *A. simplex* s.s.: *A. pegreffii*, $p = 0.077$; hybrid, $p = 0.25$), “season” ($p < 0.001$: ref. autumn; winter, $p = 0.063$; spring, $p = 0.59$), with interaction between the two ($p = 0.011$: winter $\times$ *A. pegreffii*, OR = 9.1, $p = 0.030$; winter $\times$ hybrids, $p = 1.0$; spring $\times$ *A. pegreffii*, $p = 0.99$; spring $\times$ hybrids, $p = 0.30$) and “weight” ($p = 0.006$, OR = 0.98).

3.4.4. Factors associated with the presence of the larva in the dorsal musculature

The univariate analysis shows association with the total number of larvae in the blue whiting ($p < 0.001$), genotype ($p = 0.018$) and season ($p = 0.010$). There is no interaction between these last two variables ($p = 0.82$). The length value is closer to significance ($p = 0.17$), so it was taken into account in the construction of the multivariate model.

The final multivariate model consisted of the variables “season” ($p = 0.036$: ref. autumn; winter $p = 0.097$; spring OR = 6.1, $p = 0.016$) and “total number of L3 larvae” (OR = 1.0, $p = 0.005$).

3.4.5. Factors associated with the parasitization of *A. pegreffii* in comparison with *A. simplex* s.s.

The univariate analysis shows association with the weight of the blue whiting ($p < 0.001$), total number of L3 larvae in the fish ($p < 0.001$), capture season ($p < 0.001$), location of the larva, $p < 0.001$, and the interaction of season $\times$ location ($p = 0.005$).

The final multivariate model consists of total number of L3 larvae ($p < 0.001$, OR = 0.96); and capture season ($p = 0.017$, ref. autumn; winter, $p = 0.011$, OR = 0.033; spring, $p = 0.49$), with interaction of the latter variable with location ($p = 0.013$: winter $\times$ ventral musculature, $p = 0.067$, OR = 19).

4. Discussion

The Gulf of Cadiz is a place of great ecological interest due to the confluence of warm Mediterranean and cold Atlantic waters. Many species of marine mammals that live or pass through there can act as definitive hosts to *Anisakis* spp. (Mattiucci and Nascetti, 2008), with the consequent repercussions on the parasitization of fish which act as intermediate hosts. In this study, we found a total prevalence of parasitization by *A. simplex* s.l. of 82% in *M. poutassou*, a fish in high demand in southern Europe. In blue whiting from the Atlantic Ocean prevalence values of 85% to 100% have been reported (Chía et al., 2010; Fernández et al., 2005; Viu et al., 1996); whilst the prevalence values recorded in the Mediterranean Sea for this species are lower, as reported by some

authors, who found values of 66.7% (Piras et al., 2014), 19.0% (Madrid et al., 2012) and 10.6% (Valero et al., 2000). The prevalence value found in this study is closer to those cited for Atlantic waters. On the other hand the mean abundance value of 13.5 parasites/host falls in between those found for Atlantic and Mediterranean waters. Thus, on the Mediterranean coast of North Africa, a mean abundance of *Anisakis* of 0.92 for this host was recorded (Farjallah et al., 2008), and, on the Mediterranean coast of Motril (Spain), of 0.14 (Valero et al., 2000). These values are low compared to those found on the Atlantic coast, where a mean abundance of 55.89 was reported (Chía et al., 2010). A similar scenario can be observed with regard to the presence of these nematodes in the musculature: the prevalence of 38% detected in this study is similar to 37.0% (Chía et al., 2010) and 39% (Madrid et al., 2012), in fish in the Atlantic; in contrast to the 7% found on Mediterranean coasts (Madrid et al., 2012). However, the mean intensity of infection, 8.6, is lower than that found in Atlantic waters (24.17) (Chía et al., 2010) and is closer to the figures detected in fish on Mediterranean coasts (Madrid et al., 2012; Piras et al., 2014). Knowledge of the behaviour and movements of blue whiting is far from complete, but these intermediate epidemiological parameters, which fall in between those recorded for blue whiting in Atlantic and Mediterranean waters, may be a reflection of the migratory flow between Atlantic and Mediterranean waters of blue whiting from the Gulf of Cadiz.

The prevalences of infection obtained were high in all three seasons analyzed, reaching 100% in spring (Table 1). However, the highest mean intensity and abundance values were recorded in winter. Adult blue whiting spawn at a depth of 300–500 m over deep water during the spring and this causes their condition to vary more markedly than other species of the cod family (<http://www.fao.org/wairdocs/tan/x5952e/x5952e01.htm>). Spent fish (post-spawning) are thin and soft with smaller livers, and thus are in less demand in the marketplace. By location of the larvae in the fish, the highest prevalence of parasitization was in the viscera (73%), with high values in all three seasons analyzed, and the lowest was in the dorsal musculature, particularly in autumn. Parasitization of the ventral musculature is considerably higher than that of the dorsal musculature in all 3 seasons; in spring it became similar to that of the viscera. Thus from the health perspective, eating blue whiting is safest in autumn, especially if only the dorsal musculature is consumed (Table 1).

Our results show a greater prevalence for *A. pegreffii*: 70% of the fish were parasitized by this species, and 33% as a single parasite infection, as opposed to a prevalence of 38% for *A. simplex* s.s., and 6% as a single parasite infection (Table 3). The joint presence of the two sibling species was found in 30% of the fish, which explains the frequent presence of hybrids (prevalence of 24%).

With regard to the musculature, the prevalence values of infection with *A. simplex* s.s. and *A. pegreffii* tend to be fairly equal, with a slight predominance of the former. But above all, a marked difference was detected in the intensity of infection, with double the number of *A. simplex* s.s. to *A. pegreffii* in this location. These data are in keeping with the lower capacity of penetration attributed to *A. pegreffii* both in fish tissues and laboratory animals (Arizono et al., 2012; Quiazon et al., 2008; Romero et al., 2013; Suzuki et al., 2010).

The general parasitization of blue whiting by *A. simplex* s.l. in this area of confluence of the Atlantic and Mediterranean is not influenced by the weight or length of the fish, nor by the season, probably due to the high prevalence values during the three seasons analyzed. Other authors also found that seasonality was not associated with *Anisakis* spp. infection in pleuronectiform fishes (Marques et al., 2006; Valero et al., 2000); however, an increase in prevalence and intensity to the most active reproductive season of the host was associated (Farjallah et al., 2006). In our study, the prevalence appeared to be higher in spring, but no statistically significant differences were detected. Other authors have reported higher parasitization parameters in blue whiting of greater length (Cruz et al., 2007; Madrid et al., 2012; Valero et al., 2000) and likewise in other species of fish (Abattouy et al., 2011, 2014; Hermida et

al., 2012; Molina-Fernández et al., 2015; Rello et al., 2009). The cause of this increase has been attributed to an accumulation of these nematodes over time in older fish (Macpherson, 1978). In our study we should highlight the high mean intensity and abundance figures, mainly due to *A. simplex* s.s. in blue whiting measuring 21–21.9 cm, which are relatively small specimens of approximately 3 years of age. These figures are clearly higher than those found for blue whiting of greater length (≥ 25 cm) which are 7–8 years of age (Fig. 1; Table 3) and it is possible that these values reflect differences in migratory routes.

However when we differentiate between species of *A. simplex* s.l. or analyze specific locations within the parasite, the incidence of the factors changes. Thus the presence of *A. simplex* s.l. in the viscera varies according to genotype and season: in spring and autumn, *A. pegreffii* is nearly 3 times more common than *A. simplex* s.s. in the viscera, whilst in winter the opposite occurs, and *A. simplex* s.s. is twice as common as *A. pegreffii* in this location. The factors associated with the presence of the parasite in the ventral or dorsal musculature are different. In winter, the ventral musculature holds 9 times more *A. pegreffii* larvae than *A. simplex* s.s. larvae. In the dorsal musculature, however, the highest number of parasites (*A. simplex* s.l.) was recorded in spring (6 times more than in autumn or winter). Moreover, for every larva increasing the parasitization of the fish, the probability of it being located in the dorsal musculature increases by 1%. The hybrids displayed similar behaviour to *A. simplex* s.s. in all the locations.

On comparing the ecology of *A. pegreffii* to *A. simplex* s.s. we found that the more parasitized the fish was, the greater the ratio was of *A. simplex* s.s. to its less pathogenic sibling species. The blue whiting caught in winter were mainly parasitized by *A. simplex* s.s.; *A. pegreffii* was 97% less abundant than its sibling species in this season, but it tended to accumulate in the ventral musculature, a location in which it predominates over *A. simplex* s.s.

In summary, this study confirms that the Gulf of Cadiz is a sympatric area for the species *A. simplex* s.s. and *A. pegreffii* where, due to the mixed influence of the Atlantic and Mediterranean, the parameters of infection for blue whiting are intermediate. The parasitization rate is high, even in the musculature, where *A. simplex* s.s., the most pathogenic species, is predominant. The ecology of the two sibling species with regard to their location in the fish and the influence of the season is different.

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