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ORIGINAL PAPER

# *Hysterothylacium aduncum*, the only anisakid parasite of sardines (*Sardina pilchardus*) from the southern and eastern coasts of Spain

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**Abstract** An epidemiological study was carried out on the anisakids in sardines (*Sardina pilchardus*) from the southern (Atlantic and Mediterranean) and eastern coasts of Spain. Length of fish was from 12.2–21.0 cm. The anisakids found were identified as the third larval stage (L3) of *Hysterothylacium aduncum*, with a total prevalence of 11.85%. Prevalence within the host was 9.64% in viscera and 4.69 % in muscle. The highest infection parameters were found in fish from the east coast (western Balearic Sea) with prevalence of 25.21%, mean intensity of 2.10, and mean abundance of 0.52. No worms of the genus *Anisakis* were found in the 359 sardines analyzed.

## Introduction

*Hysterothylacium aduncum* is a very common anisakid nematode in marine fish, including many commercial species. Authors such as Køie (1993) and Navone et al. (1998) have reported that, in the lifecycle of this parasite, the adults and the fourth larval stage (L4) are mainly found in the lumen of the stomach and intestine of the fish, which act as definitive hosts, while the third larval stage (L3) is found in the mesenterium of fish and in the hemocele of a wide variety of invertebrates (Køie 1993; Marcogliese 1996). Previous studies have described the presence of L3 anisakids, mainly *H. aduncum*, in the sardine (*Sardina pilchardus*). These fish were generally acquired from local markets without considering their origin (Huang 1988; Pereira Bueno 1992; Viu et al. 1996; De la Torre Molina et al. 2000). Consequently, the present study examines the anisakid parasites of sardines from different fishing zones of the southern and eastern coasts of Spain.

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## Materials and methods

A total of 359 sardines, ranging in length from 12.2–21.0 cm (mean±SD=17.1±1.9 cm), were examined. The samples, from different ports of southern and eastern Spain (Fig. 1), were grouped into three zones—zone A: Atlantic coast of southern Spain; we studied 145 sardines from the ports of Cádiz and Barbate in the eastern Gulf of Cádiz; zone B: Mediterranean coast of southern Spain; we studied 99 sardines from the ports of Málaga, Adra, and Almería, in the northern Alborán Sea; zone C: Mediterranean coast of northeastern Spain; we studied 115 sardines from the ports of Castellón, Tarragona, Barcelona, and Roses, in the western Balearic Sea.

The sardines were acquired throughout 1999 from the main wholesale fish market in Granada (southern Spain) and their origin noted. In the laboratory, the fish were measured and dissected and the viscera separated from the muscle with both tissues undergoing digestion with a pepsin–HCl solution. The anisakids released following digestion were collected, fixed, and cleared following the procedures described in previous studies (Valero et al. 2006a, b; Rello et al. 2008, submitted). For identification of

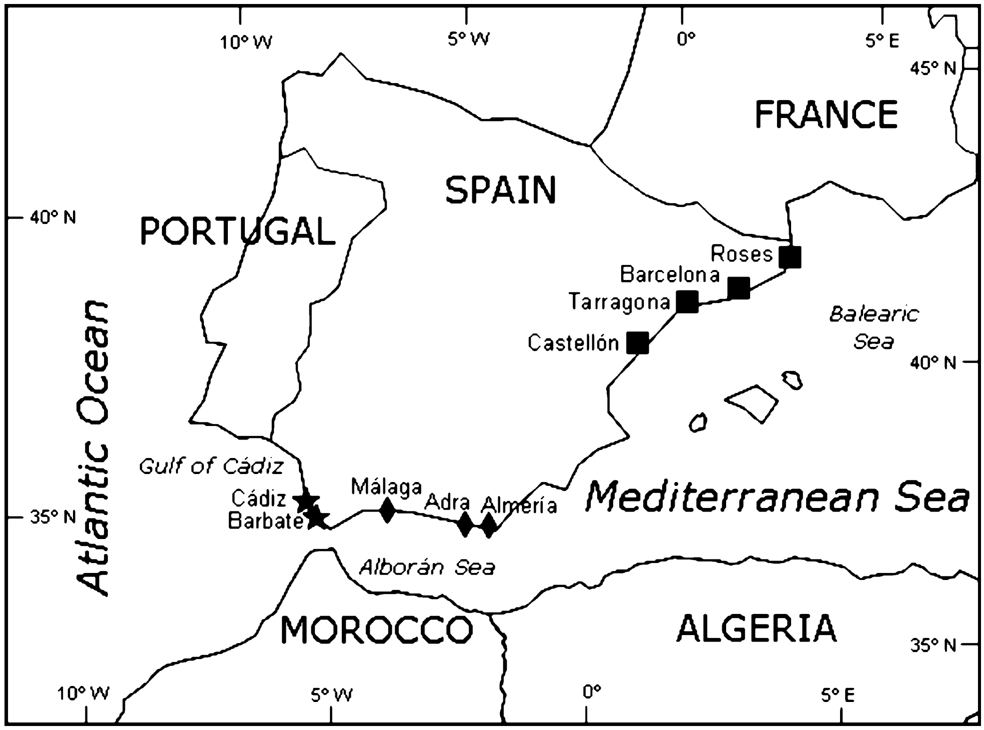


**Fig. 1** Area of investigation showing sampling ports from south and east coasts of Iberian Peninsula. *Star* zone A: Atlantic waters of southern Spain; *diamond* zone B: Mediterranean waters of southern Spain; *square* zone C: Mediterranean waters of northeastern Spain

the anisakid larvae, we followed the morphological criteria proposed by Køie (1993) and Petter and Maillard (1988).

The association between fish length and prevalence, and geographic zone and prevalence was analyzed using Fisher's exact test for comparing prevalences and bootstrap two-sample *t*-test for comparing mean intensities and mean abundances. Free QP 3.0 computer software was used for these analyses. This was developed by Reiczigel and Rózsa (2005, Quantitative Parasitology 3.0, Budapest; http://www.behav.org/qp/qp.htm) to deal with the notoriously left-biased frequency distributions of parasites, based on the theoretical background published by Rózsa et al. (2000).

## Results and discussion

The only anisakid found in this study was L3 *H. aduncum*. No *Anisakis* larvae were found, in agreement with other authors who examined sardines from several Spanish fish markets (Pereira Bueno 1992; Viu et al. 1996; De la Torre Molina et al. 2000). Several other authors also found no *Anisakis* larvae in sardines from the western Balearic Sea (zone C in the present study; Cuéllar et al. 1991; Osanz 2001). However, Huang (1988), Petter and Maillard (1988), Ruiz-Valero et al. (1992), and Sanmartín et al. (1994) all observed L3 of *Anisakis* in this host, albeit with a very low prevalence (<1.5%). The sardine feeds principally on zooplankton (mainly copepods, but also cladocerans, euphausids, crustacean larvae, anchovy eggs, …), although phytoplankton is also consumed (Cunha et al. 2005; Palomera et al. 2007 for references). The first intermediate hosts of both *Anisakis* and *H. aduncum* are small crustaceans (mainly euphausids and copepods) and other invertebrates from the zooplankton. The fact that the most frequent anisakid in sardines is *H. aduncum* and that these fish are rarely infected by *Anisakis*, although both parasites are present in the plankton, suggests that, on the one hand, the invertebrate hosts of the two parasites are different and/or, on the other hand, the euphausids from the upper layers are less parasitized by *Anisakis* than those from deep waters, resulting in less infection of pelagic clupeids (Landry et al. 1992; Osanz 2001). Nevertheless, this hypothesis does not explain the results of Silva and Eiras (2003), who reported a prevalence of *Anisakis* of 28.1% in sardines from the west coast of Portugal. This suggests that, at least in the zones studied, the ideal invertebrate hosts of *Anisakis simplex* s.l. are not present in the habitual diet of the sardine or that the sardine is not an optimal host for some species of *Anisakis*, such as *Anisakis pegreffii* (the predominant species in the Mediterranean; Mattiucci and Nascetti 2006), although it is suitable for other species such as *A. simplex* s.s. (the predominant species in the waters of the Atlantic off the western and northern coasts of the Iberian Penninsula; Mattiucci and Nascetti 2006). This would explain both our data and those of Silva and Eiras (2003). It is worth noting that, in previous studies carried out in anchovies, a pelagic coastal fish with similar feeding habits to the sardine, a prevalence of *Anisakis* (larval type I) of 13%, was found in the zone of the Gulf of Cádiz (Rello et al. submitted), this being a sympatric area of *A. simplex* s.s. and *A. pegreffii*. In

**Table 1** Infection parameters for *H. aduncum* in sardines (*S. pilchardus*)

| Zones | Fish | | | Fish viscera | | | Fish muscle | | |
|---|---|---|---|---|---|---|---|---|---|
| | *P* (%) | *I* | *A* | *P* (%) | *I* | *A* | *P* (%) | *I* | *A* |
| A | 3.40 | 1.60 | 0.05 | 3.40 | 1.20 | 0.04 | 0.68 | 2.00 | 0.01 |
| B | 9.09 | 2.56 | 0.23 | 8.00 | 1.75 | 0.14 | 4.04 | 2.25 | 0.09 |
| C | 25.21 | 2.10 | 0.52 | 18.26 | 2.05 | 0.37 | 11.30 | 1.38 | 0.16 |
| Total | 11.85 | 2.14 | 0.25 | 9.64 | 1.80 | 0.18 | 4.96 | 1.56 | 0.08 |

*P* prevalence; *I* mean intensity; *A* mean abundance

none of these studies in sardines and anchovies was genetic identification of the *Anisakis* larvae carried out.

The morphobiometric measurements of the L3 of *H. aduncum* collected showed a wide variation: 4,650–9,858 µm in length (mean±SD=6,678±1664 µm) and 103–276 µm in maximum width (mean±SD=159±45 µm; *n*=30). This variability has also been observed by several authors for the L3 collected from other host species (Petter and Maillard 1988; Ruiz-Valero et al. 1992; Navone et al. 1998). The latter suggested that these differences could depend on the degree of development attained by the larva in the previous host.

The presence of *H. aduncum* could have both economic and public health implications. Although this parasite is considered to be only mildly pathogenic for adult fish, it is highly pathogenic, leading to high mortality, for herring larvae (Balbuena el al. 2000) and young farmed halibut (Bristow 1990). Furthermore, it has been found to be present in cultures of marine fish, showing a progressive increase in prevalence with time (González and Carvajal 1994; González 1998; Sepúlveda et al. 2004). It can also affect human health. Yagi et al. (1996) described a case of noninvasive anisakiasis in a patient with diarrhea associated with the presence of an immature adult of this parasite. Fernández-Caldas et al. (1998) and Valero et al. (2003) demonstrated that this parasite has antigens in common with *A. simplex*, which may have important health implications regarding allergic processes resulting from ingestion of fish parasitized by these anisakids. It is thus useful to determine the prevalence and mean intensity in the muscle tissue of the sardine (Table 1), these being 4.96% and 1.56, respectively, with maximum prevalence in zone C (11.30%) and maximum mean intensity in zone B (2.25).

Although total prevalence was 11.85% (Table 1), it varied significantly between zones. Fish from the north-eastern coast of Spain (zone C) showed the highest prevalence (25.21%) with significances of $P$=0.002 and $P$<0.001 with respect to the fish of zones B and A. The mean abundance of *H. aduncum* was also higher in zone C than in the other zones studied (Table 1). These data are similar to those obtained by Cuéllar et al. (1991), who reported a prevalence of 29.6% for *Hysterothylacium* sp., probably *H. aduncum*, in the southwestern Balearic Sea. The greater prevalence and abundance of this worm in zone C is in agreement with the data obtained for anchovies (*Engraulis encrasicolus*) from the waters of the south and east of the Iberian Peninsula (Rello et al. submitted). The variation in the prevalence of *H. aduncum* in the fish from the different zones studied may be related to the abundance of the parasite and the presence of both invertebrate intermediate hosts and fish final hosts, as previously suggested in the studies on anchovies (Rello et al. submitted). Higher prevalences have been reported with other coasts of the Iberian Peninsula (40% in sardines caught in waters of the NW Iberian Peninsula; Sanmartín et al. 1994).

Analysis of the infection parameters of zone C (highest parasitization) reveals a tendency to increase with increasing length of the sardine, although this is not significant (Fig. 2). This tendency in the prevalence has also been reported for other fish species and, in many cases, was also statistically

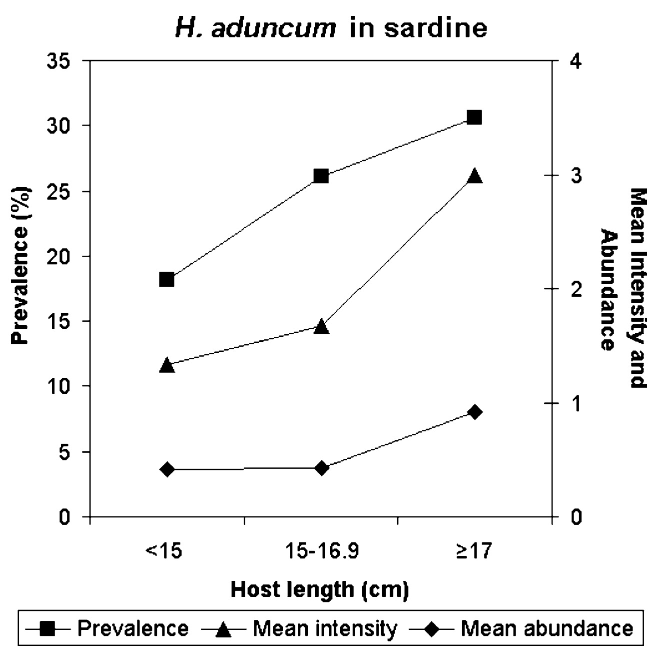


**Fig. 2** Infection parameters for *H. aduncum* by length of sardine (*S. pilchardus*) in the western Balearic Sea (northeastern coast of Spain, zone C). The number of hosts examined per length class was: 33 (<15 cm), 46 (15.0–16.9 cm), and 36 (≥17 cm)

significant (Takao 1990; Adroher et al. 1996; Hemmingsen et al. 2000; Valero et al. 2000, 2006a, b). The low prevalence of the parasite in the youngest sardines may be due to the fish larvae, at least in the Gulf of Lyon, feeding preferentially on phytoplankton (Rasoanarivo et al. 1991; Palomera et al. 2007 for references), and, consequently, the youngest fish sent to market have had less opportunity than the longer ones to become infected. In addition to the mortality of fish larvae caused by L3 of *H. aduncum* (Balbuena et al. 2000), the stress experienced by parasitized fish makes them more susceptible to other infections (González and Carvajal 1994). These weakened fish become easy prey for their predators, and, thus, the parasitized population available for sampling is reduced. Finally, the greater parasitization in the larger fish could be due to the cumulative effect of frequent ingestion of prey with parasites (Bussmann and Ehrich 1979).

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