

Anisakis spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization

S. Mattiucci^{a,*}, V. Farina^{a,b}, N. Campbell^c, K. MacKenzie^c,
P. Ramos^d, A.L. Pinto^d, P. Abaunza^e, G. Nascetti^b

^a Department of Public Health Sciences, Section of Parasitology, University of Rome, “La Sapienza”, P.le Aldo Moro, 5 00185 Rome, Italy

^b Department of Ecology and Economic Sustainable Development (DECOS), Tuscia University, Via S. Giovanni Decollato, 01100 Viterbo, Italy

^c School of Biological Sciences (Zoology), University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK

^d INIAP-IPIMAR, Avenida Brasília, 1400 Lisbon, Portugal

^e Instituto Español de Oceanografía, Centro Oceanográfico de Santander, Apdo. 240, 39080 Santander, Spain

Abstract

In the present study, data are reported on the use of *Anisakis* spp. larvae, genetically identified to species level, for stock identification of Atlantic horse mackerel, *Trachurus trachurus*, throughout its range. Genetic markers applied to 2200 *Anisakis* larvae, allowed the detection of *Anisakis simplex* (s.s.), *Anisakis pegreffii*, *Anisakis physeteris*, *Anisakis typica* and *Anisakis* sp., in 20 horse mackerel samples. The two most dominant species were *A. pegreffii* and *A. simplex* (s.s.), with statistically significant differences between samples. *A. simplex* (s.s.) was the most common species found in all samples from NE Atlantic waters, with its relative proportion decreasing from north (south coast of Norway) to south (Portuguese coast off Algarve). In contrast, *A. pegreffii* was the most prevalent species in the Mediterranean Sea. Mixed infections of *A. simplex* (s.s.) and *A. pegreffii* were found in all samples from the Portuguese and Spanish Atlantic coasts and in a sample from south of Ireland. Finally, in horse mackerel caught off Málaga (Mediterranean Sea), mixed infections of the same two species were found in almost equal proportions. *Anisakis physeteris* occurred rarely and in mixed infections with *A. pegreffii* in a sample from the Tyrrhenian Sea. Similarly, *A. typica* was rarely found in mixed infections with *A. pegreffii* in the eastern Mediterranean (Aegean Sea), as well as the *Anisakis* sp. with *A. simplex* (s.s.) in the North Sea samples. The biogeographical pattern of *Anisakis* spp. larvae indicates the presence of distinct Mediterranean and Atlantic stocks of horse mackerel and supports the existence of a “northern” stock separated from a “southern” one in the Atlantic region. The results also highlight the vagility of horse mackerel, supporting the hypothesis of some migration from the Atlantic into the extreme Western Mediterranean Sea.

© 2007 Elsevier B.V. All rights reserved.

Keywords: *Trachurus trachurus*; *Anisakis* spp.; Genetic markers; Biological tags; Stock identification; Mediterranean; NE Atlantic

1. Introduction

Multilocus enzyme electrophoresis has been widely used in the last two decades to investigate patterns of genetic variation in a wide range of anisakid nematode populations belonging to the genus *Anisakis* Dujardin, 1845, a cosmopolitan endoparasite, in its adult stage, of various cetacean species and, in its larval stages, of various invertebrate (crustacean and cephalopod) and fish hosts. The main questions best answered by the application of these genetic markers to this group of parasitic nematodes were concerning (a) the clarification of the

taxonomy of the species belonging to this genus; (b) the identification of their larval stages, which are also the causal agents of human disease (anisakidosis); (c) the collection of ecological data regarding their geographical distribution and the definitive and intermediate/paratenic hosts involved in their life-cycles; (d) the estimation of their genetic differentiation and relationships (Nascetti et al., 1986; Mattiucci et al., 1986, 1997, 1998, 2001, 2002, 2004, 2005). The genus *Anisakis*, earlier considered to include only three valid species as reviewed by Davey (1971), has been revised by the application of allozyme markers. Their use has (i) detected sibling species within some of the morphospecies recognized as valid in Davey’s revision (e.g. three sibling species within *A. simplex* s.l., a species in the past considered as cosmopolitan); (ii) validated the taxonomic status of *A. brevispiculata*, considered by Davey (1971) as a synonym

* Corresponding author. Tel.: +39 0649914894; fax: +39 0649914894.
E-mail address: simonetta.mattiucci@uniroma1.it (S. Mattiucci).

of *A. physeteris*, from which reproductive isolation in sympatry was demonstrated (Mattiucci et al., 2001); (iii) identified new species belonging to the genus, such as *A. ziphidarum*, a parasite of beaked whales (Paggi et al., 1998), and *A. paggiae*, a parasite of the dwarf and pigmy sperm whales (Mattiucci et al., 2005); (iv) identified the larval stages within the genus, revealing that *A. simplex* (s.s.), *A. pegreffii*, *A. simplex* C, *A. typica*, *A. ziphidarum* share the morphotype Type I (*sensu* Berland, 1961), whereas *A. physeteris*, *A. brevispiculata*, and *A. paggiae* (Mattiucci et al., 2005) show the morphotype Type II (Berland, 1961; Mattiucci et al., 2002, 2004, 2005).

The last surprising results, arising from the work carried out using allozyme markers, have been revealed to have a fruitful application to the recognition of different *Anisakis* spp., at any life-stage and in both sexes, which can often be found in sympatry and syntopy infecting the same host species, both definitive (cetaceans) or intermediate (fish and/or squid). This last application proved to be a powerful tool in the larval identification of seven species of *Anisakis*, showing different patterns of distribution in the same fish host, the European hake (*Merluccius merluccius* L.), throughout its geographic range, thereby allowing us to distinguish its stocks in Atlantic and Mediterranean waters (Mattiucci et al., 2004).

The purposes of this paper were to (i) determine, at species level, by the use of genetic markers, which species of *Anisakis* parasitize the Atlantic horse mackerel, *Trachurus trachurus*, throughout its geographic distribution; (ii) analyse the patterns of distribution of *Anisakis* spp. in horse mackerel samples to verify the existence of different Atlantic horse mackerel stocks.

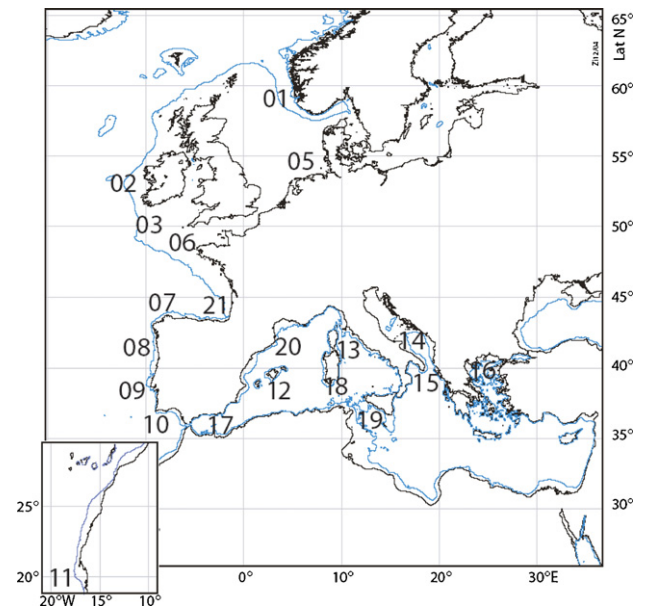


Fig. 1. Sampling localities of *Trachurus trachurus* examined for *Anisakis* larvae.

2. Materials

A total of 2200 *Anisakis* spp. larvae collected from all the fishing areas (Fig. 1) comprising the geographical range of the fish host were identified to species level, by means of genetic markers obtained from multilocus allozyme electrophoresis. Table 1 shows the number of larvae analysed from each locality. The larvae, after their removal from each fish, were first washed in

Table 1

Relative proportions of the *Anisakis* spp. identified by allozyme markers in horse mackerels from the sampling localities

Sampling locality	N_p	<i>A. pegreffii</i>	<i>A. simplex</i> (s.s.)	<i>Anisakis</i> sp.	<i>A. physeteris</i>	<i>A. typica</i>
North East Atlantic Ocean						
01. Southwest Norway	154	–	100.0	–	–	–
02. West of Ireland	142	2.1	97.9	–	–	–
03. Great Sole Bank (South of Ireland)	99	2.0	98.0	–	–	–
21. Southern Bay of Biscay	80	1.3	98.7	–	–	–
05. North Sea	91	–	98.0	2.0	–	–
06. Western English Channel	92	–	100.0	–	–	–
07. Northwest of Spain (Galicia)	85	29.4	70.6	–	–	–
08. North of Portugal	248	38.7	61.3	–	–	–
09. Central Portugal	109	51.4	48.6	–	–	–
10. South of Portugal	141	86.5	13.5	–	–	–
P_1		*** (1)	** (2)	NC		
Mediterranean Sea						
13. Tyrrhenian Sea	129	99.0	–	–	1.0	–
14. Adriatic Sea	116	100.0	–	–	–	–
15. Ionian Sea	129	100.0	–	–	–	–
16. Aegean Sea	115	99.0	–	–	–	1.0
17. Alborán Sea (Málaga)	195	48.7	51.3	–	–	–
18. South Sardinian Sea	132	100.0	–	–	–	–
19. South of Sicily	138	100.0	–	–	–	–
P_1		NS	NC			
P_2		*** (1)	*** (1)			

P : probability level of the statistical significance of the comparison among number of *Anisakis pegreffii* and *A. simplex* s.s. identified in North Atlantic horse mackerels (P_1) and in Mediterranean and North Atlantic horse mackerels (P_2); obtained from χ^2 test⁽¹⁾ and χ^2 (Yates corrected)⁽²⁾; *0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001; NC: not calculated; NS: not significant.

saline solution, then stored frozen at -70°C , in Eppendorf tubes, until their genetic analysis.

3. Methods

The procedures for multilocus allozyme electrophoresis were those reported in Nascetti et al. (1986) and Mattiucci et al. (1997). Among the 20 enzyme-loci studied in *Anisakis* spp. so far (Mattiucci et al., 1997, 1998, 2001, 2002, 2005) the following were used to identify the larvae to species: malate dehydrogenase (*Mdh-1*, EC 1.1.1.37), superoxide dismutase (*Sod-1*, EC 1.15.1.1), nucleoside phosphorylase (*Np*, EC 2.4.2.1), aspartate amino transferase (*Aat-2*, EC 2.6.1.1), adenylate kinase (*Adk-2*, EC 2.7.4.3), leucine aminopeptidase (*Lap-1*, EC 3.4.11.1), leucine–leucine–leucine peptidase (*PepB*, EC 3.4.11) and leucine–alanin peptidase (*PepC-1*, *PepC-2*, EC 3.4.11), mannose phosphate isomerase (*Mpi*, EC 5.3.1.8), glucose phosphate isomerase (*Gpi*, EC 5.3.1.9) and phosphoglucomutase (*Pgm-2*, EC 5.4.2.2). Their staining procedures are reported, in detail, in Mattiucci et al. (1997). The genetic structure of larval populations from all the selected localities studied was compared with those from all the *Anisakis* spp. so far genetically detected and characterized (Mattiucci et al., 1997, 2001, 2002, 2005). The allozymes found in the previously characterized species of *Anisakis*, were used for the identification of all the 2200 larvae from *T. trachurus* tested. All the larvae recovered were genetically identified except from the localities 01, 02, 21, 05, 06, 13, 14, 15, 16, 18 and 19; from these areas, a subsample (not less than 100 larvae) of all *Anisakis* collected from each infected fish were identified by the allozyme markers. Population genetics analysis was carried out by BIOSYS 2.1 software (Swofford and Selander, 1989). Statistical significance of the differences observed in the relative proportions of the *Anisakis* spp. identified between sampling localities was tested by the χ^2 -test and Fisher's exact test, according to Rószsa et al. (2000). Differences were considered to be significant when $P < 0.05$.

4. Results

4.1. Species of *Anisakis* identified by allozyme markers in horse mackerel

The *Anisakis* larvae collected from the sampling localities of *T. trachurus* were morphologically ascribed to the morphotypes indicated as *Anisakis* Type I and Type II (*sensu* Berland, 1961): a few Type II larvae were collected only from locality 13, in the Tyrrhenian Sea. The diagnostic loci detected among the species of *Anisakis* so far genetically studied (see Mattiucci et al., 2002, 2005), permitted the identification of at least five taxa: *A. simplex* (s.s.), *A. pegreffii*, *A. physeteris*, *A. typica* and *Anisakis* sp. (Table 1).

4.2. Relative proportions of *Anisakis* spp. in horse mackerel

Of the five species of *Anisakis* identified in *T. trachurus*, the two sibling species *A. pegreffii* and *A. simplex* (s.s.) were found to be the dominant species in all the fish samples, whereas *A. phy-*

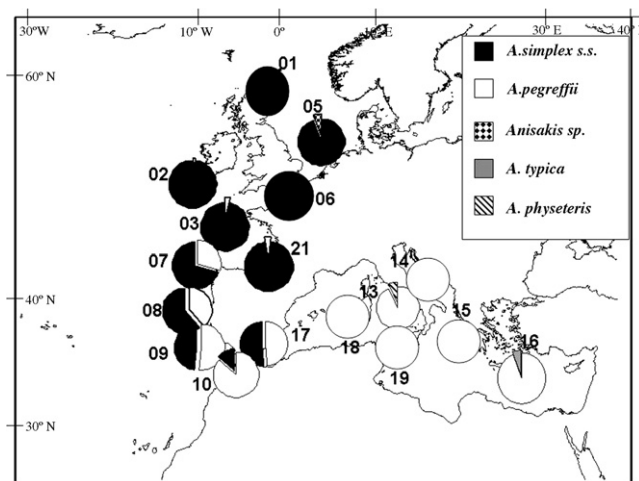


Fig. 2. Distribution patterns of *Anisakis* spp. larvae identified by genetic markers in *T. trachurus* from all the selected localities.

seteris and *A. typica* were identified only at very low percentages (Table 1). *A. pegreffii* and *A. simplex* (s.s.) showed statistically significant differences in their relative proportions in the horse mackerel samples throughout NE Atlantic and Mediterranean waters. Indeed, *A. pegreffii* was identified as the main species parasitizing *T. trachurus* in the Mediterranean Sea; it was the only species present from the Adriatic and Ionian Seas, with the only exception represented by the Aegean Sea where a rare occurrence (1%) of *A. typica* was detected (Table 1). Similarly, in the western Mediterranean Sea, *A. pegreffii* was the dominant species in all the fish examined; only rarely were *A. physeteris* larvae identified in horse mackerel caught along the Tyrrhenian coast (Table 1). Interestingly, the samples of *T. trachurus* caught in the Alborán Sea (Mediterranean Gibraltar area) showed mixed infections by both the sibling species, *A. pegreffii* and *A. simplex* (s.s.), in almost equal proportions (Table 1 and Fig. 2). Moving to the Atlantic samples of *T. trachurus*, *A. pegreffii* showed a progressively decreasing proportion of occurrence, ranging from 86.5% observed in fish from the Atlantic Portuguese coast off Algarve to 51.4% reported in those from the central Portuguese coast, to 38.7% in those from the north of Portugal and, finally, to 29.4% in the sample from the Spanish Galician coast. *A. pegreffii* was also identified rarely (range 1.3–2.1%), in the samples from southern and western waters of Ireland (Table 1 and Fig. 2).

Conversely, in the same individual fish from Atlantic waters, *A. simplex* (s.s.) showed an increasing proportion from the samples caught off the south coast of the Iberian Peninsula (Portuguese coast off Algarve), to the Atlantic Galician coast of Spain (Table 1). Indeed, its relative proportions ranged from 13.5% to 70.6% in Atlantic horse mackerel. It was also the dominant species in *T. trachurus* from the west coast of Ireland (97.9%), off the south coast of Ireland (Great Sole Bank) (98.0%), and from the Bay of Biscay (98.7%). It became the only species present in the fish from the North-East Atlantic, reaching 100% in the Norwegian Sea and along the western English Channel (Table 1 and Fig. 2).

Finally, the horse mackerel from the Morocco-Mauritanian coast were not found to be heavily infected by *Anisakis* spp.

larvae: of the only six larvae recovered in that sample, two were identified as *A. pegreffii* and four as *A. simplex* (s.s.). In the Atlantic samples, some larvae corresponded to a new gene pool previously identified at the preadult stage from a beaked whale, *Mesoplodon mirus*, from central and south Atlantic waters and here indicated as *Anisakis* sp. (Mattiucci, data not shown).

4.3. Mixed infections of horse mackerels by *A. pegreffii* and *A. simplex* (s.s.)

The two dominant species of the *A. simplex* complex were found sympatrically occurring in almost 34% of the total number of *T. trachurus* examined in the present study. Mixed infections by the two sibling species *A. pegreffii* and *A. simplex* (s.s.) were found in the same individual hosts caught along the Iberian coast (Atlantic coast) and in the Alborán Sea (Mediterranean coast) (Table 2). Indeed, almost 50% of the fish examined from off the north coast of Portugal (locality no. 08) were infected by both species, whereas the remainder showed infection by only one of the two sibling species of *Anisakis* (Table 2). A similar situation was detected in the Mediterranean area of the Alborán Sea where, out of the total fish examined, at least 61.5% had a double infection, with 34.6% infected by only *A. pegreffii*, and the remaining 3.9% showing single infections by *A. simplex* (s.s.). In Atlantic waters, the percentage of fish infected by only *A. simplex* (s.s.) only was almost double the percentage with single infections of *A. pegreffii*, except for those from the south coast of Portugal (off Algarve), where the number of fish showing single infections of *A. pegreffii* was 10 times greater (73.0%) than those parasitized by only *A. simplex* (s.s.) (9.5) (Table 2). Out of the six *Anisakis* larvae collected from the *T. trachurus* sample from the Mauritanian shelf (locality no. 11) were found to correspond to *A. pegreffii* ($n = 4$) and to *A. simplex* s.s. ($n = 2$); this result was not considered in Table 1 and Fig. 2 due to the small number of larvae found in this sample.

5. Discussion

5.1. Biogeographical aspects of *Anisakis* spp. larvae identified in horse mackerel

Allozyme markers from multilocus allozyme electrophoresis of *Anisakis* spp. larvae were first used to differentiate populations of herring *Clupea harengus* L. in the North Atlantic Ocean (Beverley-Burton, 1978). The results here obtained in the present study indicate that these genetic markers are very useful in the identification of larval stages of *Anisakis* spp., which lack morphological diagnostic characters at the species level. Indeed, larvae sharing the same morphology Type I (*sensu* Berland, 1961) are demonstrated in the present paper to comprise at least three biological species as parasites of the same fish host: *A. simplex* (s.s.), *A. pegreffii* and *A. typica*. Of those *Anisakis* spp. sharing the morphotype II (Mattiucci et al., 2005), the species *A. physeteris* was identified in the *T. trachurus* examined. Moreover, allozyme markers were very useful for the detection of mixed infections by these *Anisakis* species in a large number of *T. trachurus* examined.

Previous detection of *A. pegreffii* larvae in *T. trachurus* from Mediterranean Sea was by Mattiucci et al. (1997), when it proved to be the main species of *Anisakis* from all the selected localities of the Mediterranean Sea. This is in accordance with the geographical range reported so far for *A. pegreffii* (Mattiucci et al., 1997). In addition, its occurrence in Atlantic fish from waters along the Portuguese and Spanish coasts are in accordance with previous findings for *A. pegreffii*, at both the adult and larval stages (Mattiucci et al., 2004, 2005). The identification of this Atlantic area as a contact zone between the two sibling species of the *A. simplex* complex was previously demonstrated (Mattiucci et al., 1997, 2004, 2005). Although a very high percentage of fish captured showed mixed infections by both species, only 5 out of the 2200 larvae identified were F1 hybrids between the two taxa; on the other hand no F1 hybrid at the adult stage was identified in mixed infections in cetaceans from the same locality (Mattiucci et al., 2005) supporting the hypothesis of their reproductive isolation in a contact area.

Concerning *A. simplex* (s.s.), the results here obtained are in agreement with the geographical range so far reported for this species (Mattiucci et al., 1997, 1998). *Anisakis simplex* (s.s.) was previously reported by Mattiucci et al. (1997) and Abollo et al. (2001) from Atlantic horse mackerel, but its occurrence in 4.0% of this fish species in the Alborán Sea (western Mediterranean Sea) is of particular interest and is the first record from this area (Table 1). Indeed previous findings of *A. simplex* (s.s.), identified by genetic markers, from fish captured in western Mediterranean waters were from other pelagic fish species such as the mackerel *Scomber scombrus* (Nascetti et al., 1986) and the bluefin tuna *Thunnus thynnus* (Mattiucci, unpublished data).

As for *A. pegreffii* and *A. simplex* (s.s.), they were shown to be the dominant anisakid nematode species in *T. trachurus*, being the two most prevalent species in both the Mediterranean and Atlantic samples (see MacKenzie et al., 2008), so they may be regarded as common parasite species for *T. trachurus* (Table 1 and Fig. 2). This finding indicates that *T. trachurus* represents a suitable intermediate host for both these *Anisakis* species in temperate and cold waters, favouring their transmission to their main preferred definitive hosts represented by the oceanic dolphins. Indeed, both *A. simplex* (s.s.) and *A. pegreffii* have been demonstrated to show host preferences for cetacean species in Delphinidae, Monodontidae and Phocoenidae (Mattiucci et al., 1997, 2005). The present finding about the occurrence of *A. simplex* (s.s.) and *A. pegreffii* as dominant species in *T. trachurus* could be related to the feeding habits and mode of life of this fish species, which has as its main prey items pelagic crustaceans and fish (such as anchovies), rather than squids, which are also found infected by *Anisakis* larvae (Mattiucci et al., 1997). Horse mackerel is mainly a zooplanktivorous fish (Ben-Salem, 1988), which favours its infection by *Anisakis* larvae. Thus, juvenile and adult horse mackerel became infected when preying on both crustaceans and teleosts. In contrast, the demersal fish *M. merluccius* was found infected by seven different species of *Anisakis* larvae (Mattiucci et al., 2004), suggesting that this fish species is more sedentary, and with different feeding habits in the water column, thus allowing it to acquire infections by other *Anisakis* spp. also having squids as main intermediate hosts.

Table 2

Percentage of horse mackerels showing mixed infection by *A. simplex* s.s and *A. pegreffii* in European waters

Sampling locality	Single infections of <i>A. simplex</i> (s.s.)	Single infections of <i>A. pegreffii</i>	Double infection <i>A.</i> <i>pegreffii</i> – <i>A. simplex</i> (s.s.)
Northeast Atlantic Ocean			
02. West of Ireland	93.7	–	6.3
03. Great Sole Bank (South of Ireland)	94.7	–	5.3
21. Southern Bay of Biscay	95.8	–	4.2
07. Northwest of Spain (Galicia)	50.0	15.6	34.4
08. North of Portugal	42.0	14.8	43.2
09. Central Portugal	39.3	28.6	32.1
10. Southern Portugal	9.5	73.0	17.5
11. Mauritanian shelf	67.7	33.3	–
Mediterranean Sea			
17. Alborán Sea (Málaga)	3.9	34.6	61.5

Species of the genus *Trachurus* are a prey species group for some cetaceans, for example, the common dolphin, *Delphinus delphis*, in Atlantic Iberian waters (Silva, 1999). This cetacean species was also demonstrated to be a suitable definitive host for both *A. pegreffii* and *A. simplex* (s.s.) in Spanish Atlantic waters (Mattiucci et al., 2005). The occurrence of some *T. trachurus* caught at Great Sole Bank (locality no. 03) and from west of Ireland (locality no. 02) parasitized (5.3%) (Table 2) by both *A. pegreffii* and *A. simplex* (s.s.) could have a different explanation. This phenomenon was already evidenced also in the European hake (*M. merluccius*) from the same collection localities. In the case of horse mackerel it could be correlated with the migration of the adult fish from off Galicia to the Great Sole Bank or to the southern eastern part of Ireland. Moreover, Galician waters and southern Ireland are included in the geographical range in the Atlantic Ocean of cetaceans (López et al., 2001) which are also the definitive hosts of these two *Anisakis* species in the same waters (Mattiucci et al., 2005).

The two species *A. typica* and *A. physeteris* could be considered as rare parasite species of *T. trachurus*, being present in low numbers in the horse mackerel examined (Table 1 and Fig. 2). *Anisakis physeteris* was previously reported in this fish host from the Mediterranean Sea by Mattiucci et al. (2001). It is likely that squids rather than fish are its main intermediate hosts, these also being the main prey items of the sperm whale, *Physeter macrocephalus* (Santos et al., 2002), the main definitive host of *A. physeteris* in both Mediterranean and Atlantic waters (Mattiucci et al., 2001).

As regards *A. typica*, its low occurrence in the fish examined could be related to the fact that the selected localities for horse mackerel sampling do not correspond to the geographical range so far reported for the species *A. typica* (Mattiucci et al., 2002, 2004). However, its presence in the eastern part of the Mediterranean (Aegean Sea) seems to be in accordance with previous findings in a demersal fish species, the European hake, *M. merluccius*, from the same area of the Mediterranean Sea (Mattiucci et al., 2004). This being the species detected in cetaceans and fish hosts from tropical and temperate-tropical regions (Mattiucci et al., 2002, 2004, 2005), it was considered that its presence in waters of this basin could be a consequence of the migration of other intermediate and/or definitive hosts

from the Indian Ocean to the Mediterranean through the Suez Channel (Lessepsian migration; Por, 1978).

5.2. *Anisakis* spp. larvae as “biological tags” for horse mackerel stocks in European waters

Different populations of *T. trachurus* can be distinguished according to the genetically recognized species of *Anisakis*, and from their biogeography and life-cycle aspects. Indeed, the statistically significant differences evidenced in the relative proportions of the two sibling species *A. simplex* (s.s.) and *A. pegreffii* between the samples from the Mediterranean Sea (excluding the Alborán Sea) and those from the Atlantic (Table 1), seems to indicate a discrete sub-structuring of populations of *T. trachurus* in Mediterranean and Atlantic waters (Fig. 2). Moreover, in Atlantic waters, the significant differences found in the proportion of *A. pegreffii* between the southern horse mackerel samples from the fishing areas represented by samples 07, 08, 09, 10 and 11, and those remaining from the Atlantic, seem to suggest a possible existence of a “southern” subpopulation of *T. trachurus* readily distinguished from a “northern” one represented by samples 03, 05 and 21 (Fig. 2). According to the *Anisakis* spp. composition observed, the samples of *T. trachurus* from the Alborán Sea, were more similar to the southern populations of the Atlantic than to the other samples from the Mediterranean Sea. This finding, and particularly the presence of *A. simplex* (s.s.) in some individual hosts (Table 2), suggests that these populations could be the result of a migration of this small pelagic fish species from Atlantic waters into this extreme western area of the Mediterranean Sea, also possibly mixing with Mediterranean populations. The presence of *A. simplex* (s.s.) in the Gibraltar area of the Alborán Sea was previously reported in other pelagic fish species (i.e. *Scomber scombrus* and *Thunnus thynnus*) (Mattiucci et al., 1997; unpublished data). The migration of Atlantic horse mackerel into the western part of the Mediterranean Sea is also supported by the finding of the monogenean *Heteraxinoides atlanticus* in this sample (MacKenzie et al., 2008). Thus, the high percentage of fish showing infection by *A. simplex* (s.s.) only is in accordance with the high vagility of *T. trachurus*. This is, in turn, also in agreement with the high

genetic variability values evidenced for *T. trachurus*, at the nuclear level (allozymes) (Cimmaruta et al., 2008).

Moreover, the finding of a small percentage of *A. pegreffii* in localities 02 and 03 (southern part of Ireland and Great Sole Bank) seems to indicate that a mixing of *T. trachurus* populations also occurs in this area. The occurrence of *A. pegreffii* at a small percentage was also reported in European hake captured in the same area of the NE Atlantic (Mattiucci et al., 2004), suggesting that both Galician waters and those off southern Ireland are closer than the south-eastern part of the Bay of Biscay to the distribution of cetaceans, the definitive hosts of these parasite species in the Atlantic Ocean (Cousteau and Paccalet, 1986; López et al., 2001).

6. Conclusions

- (i) The present study, based only on the *Anisakis* species composition, supports the characterization of a subpopulation structure of *T. trachurus* in European waters: the eastern and central Mediterranean populations seem to be largely separated from the Atlantic ones. The Alborán Sea (western Mediterranean Sea) population is more similar to that in the southern part of the Atlantic area of this study than to the other Mediterranean populations, or consists of a mixture between them. In the NE Atlantic Ocean, there appears to be a “northern stock” and a “southern stock”.
- (ii) The findings suggest that *Anisakis* species could be useful indicators of the biology of a fish species, in relation to its migration route and feeding habits.
- (iii) The results confirm that *Anisakis* species also represent good biological tags to be used within a multidisciplinary approach (Waldman, 1999) in “fish stock assessment” studies.
- (iv) The present study validates the genetic markers, obtained from multilocus allozyme electrophoresis, as a precise tool in the identification of a high number of larval specimens of these anisakid nematodes.

Acknowledgements

The authors gratefully acknowledge two anonymous referees whose suggestions and comments greatly improved the MS. We also thank Jeremy W. Lichstein from the “Cooperative Fish and Wildlife Research Unit”, Department of Zoology, North Carolina State University, for critically reading the manuscript, and for suggestion in statistical analysis of the data. This study was granted by EC – QLK5 Ct-1999-01438.

References

- Abollo, E., Gestal, C., Pascual, S., 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitol. Res.* 87, 492–499.
- Ben-Salem, M., 1988. Régime alimentaire de *Trachurus trachurus* (Linnaeus, 1758) et de *T. mediterraneus* (Steindachner, 1868). (Poissons, Téléostéens, carangidae) de la province Atlantico-Méditerranéenne. *Cybiu* 12, 247–253.
- Berland, B., 1961. Nematodes from some Norwegian marine fishes. *Sarsia* 2, 1–50.
- Beverley-Burton, M., 1978. Population genetics of *Anisakis simplex* (Nematoda: Ascaridoidea) in Atlantic salmon (*Salmo salar*) and their use as biological indicators of host stocks. *Environ. Biol. Fishes* 3, 369–377.
- Cimmaruta, R., Bondanelli, P., Ruggi, A., Nascetti, G., 2007. Genetic structure and temporal stability in horse mackerel (*Trachurus trachurus*). *Fish. Res.*, this issue.
- Cousteau, J.Y., Paccalet, Y., 1986. Whales. Arry N. Abrams Inc., New York, p. 280.
- Davey, J.T., 1971. A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridida). *J. Helminthol.* 45, 51–72.
- López, A., Santos, M.B., Pierce, G.J., González, A.F., Valeiras, X., Guerra, A., 2001. Trends in stranding and by-catch marine mammals in north-west Spain during the 1990s. *J. Mar. Biol. Ass. U.K.* 82, 513–521.
- MacKenzie, K., Campbell, N., Mattiucci, S., Ramos, P., Pinto, A.L., Abaunza, P., 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. *Fish. Res.* 89, 136–145.
- Mattiucci, S., Abaunza, P., Ramadori, L., Nascetti, G., 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. *J. Fish Biol.* 65, 495–510.
- Mattiucci, S., Nascetti, G., Bullini, L., Orecchia, P., Paggi, L., 1986. Genetic structure of *Anisakis physeteris* and its differentiation from the *Anisakis simplex* complex (Ascaridida: Anisakidae). *Parasitology* 93, 383–387.
- Mattiucci, S., Nascetti, G., Cianchi, R., Paggi, L., Arduino, P., Margolis, L., Brattey, J., Webb, S.C., D’Amelio, S., Orecchia, P., Bullini, L., 1997. Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *J. Parasitol.* 83, 401–416.
- Mattiucci, S., Nascetti, G., Dailey, M., Webb, S.C., Barros, N., Cianchi, R., Bullini, L., 2005. Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nematoda: Anisakidae). *Syst. Parasitol.* 61, 157–171.
- Mattiucci, S., Paggi, L., Nascetti, G., Abollo, E., Webb, S.C., Pascual, S., Cianchi, R., Bullini, L., 2001. Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). *Int. J. Parasitol.* 31, 9–14.
- Mattiucci, S., Paggi, L., Nascetti, G., Ishikura, H., Kichuki, K., Sato, N., Cianchi, R., Bullini, L., 1998. Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda Ascaridoidea). *Syst. Parasitol.* 40, 81–92.
- Mattiucci, S., Paggi, L., Nascetti, G., Portes Santos, C., Costa, G., Di Benedetto, A.P., Ramos, R., Argyrou, M., Cianchi, R., Bullini, L., 2002. Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). *Syst. Parasitol.* 51, 159–170.
- Nascetti, G., Paggi, L., Orecchia, P., Smith, J.W., Mattiucci, S., Bullini, L., 1986. Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North East Atlantic. *Int. J. Parasitol.* 16, 633–640.
- Paggi, L., Nascetti, G., Webb, S.C., Mattiucci, S., Cianchi, R., Bullini, L., 1998. A new species of *Anisakis* Dujardin, 1845 (Nematoda Anisakidae) from beaked whales (Ziphiidae): allozyme and morphological evidence. *Syst. Parasitol.* 40, 161–174.
- Por, D.F., 1978. Lessepsian Migration. Springer Verlag, New York.
- Róza, L., Reiczig, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. *J. Parasitol.* 86, 228–232.
- Santos, M.B., Pierce, G.J., Hartmann, M.G., Smeenk, C., Addink, N., Kuiken, T., Reid, R.J., Patterson, I.A.P., Lordan, C., Rogan, E., Mente, E., 2002. Additional notes on stomach contents of sperm whale *Physeter macrocephalus* stranded in the north-east Atlantic. *J. Mar. Biol. Ass. U.K.* 82, 501–507.
- Silva, M.A., 1999. Diet of common dolphins, *Delphinus delphis*, off the Portuguese continental coast. *J. Mar. Biol. Ass. U.K.* 79, 531–540.
- Swofford, D.L., Selander, R.B., 1989. BYOSYS-1, A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics, Version 1.7. Natural History Survey, Champaign, IL, p. 31.
- Waldman, J.R., 1999. The importance of comparative studies in stock analysis. *Fish. Res.* 43, 237–246.