

RESEARCH NOTE

# Helminth parasites of the Atlantic chub mackerel, *Scomber colias* Gmelin, 1789 from Canary Islands, Central North Atlantic, with comments on their relations with other Atlantic regions

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## Abstract

Eleven parasite taxa were found infecting 68 Atlantic chub mackerel, *Scomber colias* Gmelin, 1789 from the Canary Islands, Central North Atlantic. The most abundant parasites were the gill monogenean *Pseudokuhnia minor* (P = 54.4%), larval anisakid nematodes (P = 11.8%) in the body cavity, a larval tetraphyllidean infecting bile ducts (P = 8.8%) and didymozoid digeneans infecting the gills (P = 7.4%). No correlation between fish length and abundance of infection with these parasites was found. Within the Atlantic, the comparison of present results with previous reports on the occurrence of parasites in this fish host, might suggest that there is more than one population unit of Atlantic chub mackerel in the Eastern Atlantic.

## Keywords

*Scomber colias*, fish, Canary Islands, helminth parasites

Despite the absence of physical barriers in the marine realm, closely related marine fish species may show differences in spawning behaviour, life-history traits and growth rates that could reveal a distinct population structuring (Knutzen *et al.* 2003). The Atlantic chub mackerel, *Scomber colias* Gmelin, 1789 (Scombridae), a commercially important fish species, is an example of a pelagic marine fish whose systematic status could be controversial due to the existence of geographically distant populations with morphological variability and wide distributional pattern, encompassing the North and South Atlantic, as well as the Mediterranean Sea (Collette and Nauen 1983). It is important as a fishing resource and as a component of the diet of larger pelagic fish, sharks, as well as marine mammals (dolphins and whales) (Lockwood 1988). Scoles *et al.* (1998) considered the existence of a single stock of chub mackerel in the Atlantic based on mtDNA analysis. However,

a comparison of the helminth parasite composition of this fish species from Madeira and South Atlantic (Brazil), found differences in intensity and prevalence of parasites (Oliva *et al.* 2008). Several authors have consistently highlighted the utility of parasitological surveys in understanding and differentiating fish populations, and have successfully demonstrated that indeed parasites are good biological tracers or tags for fish populations (MacKenzie 1990, 2002; Hemmingsen *et al.* 1991; George-Nascimento and Arancibia 1992; Williams *et al.* 1992; Santos *et al.* 2009). Investigations on the parasites of Atlantic chub mackerel from Madeira, in particular of anisakid nematodes and monogeneans, have already highlighted interesting differences in both prevalence and abundance data between Madeira and other Atlantic regions (Costa *et al.* 2003, 2007). After a survey of helminth parasites of chub mackerel caught in Madeira Archipelago (Central Northeast Atlantic)

conducted in 1999–2000 and 2004–2005 we had the opportunity of examining a sample of Atlantic chub mackerel caught from the Canary Archipelago, providing material for: (1) the characterization of the parasite composition of the Atlantic chub mackerel in that region and (2) comparing the data obtained with the present state of knowledge on the parasite fauna of this fish host within the Central North-East Atlantic, namely Madeira, Azores and the North African coast.

Sixty-eight Atlantic chub mackerel, *S. colias*, were obtained from commercial fish landings in Tenerife (Canary Islands, Spain) (28°16'7"N and 16°36'20"W) in November 2009. Of those, 20 were examined fresh and the remaining were deep-frozen at -17°C and subsequently examined after defrosting, at room temperature. In the laboratory fish were measured in centimeters (cm) and weighed in grams (g). Gills were removed and examined under a dissecting microscope for the presence of ectoparasites (monogeneans and crustaceans). Opercula were cut and examined for the presence of cysts of didymozoids. The body cavity was exposed and viscera carefully removed, placed in Petri dishes, and thereafter examined under a dissecting microscope, for the presence of helminth parasites. Stomach and intestine were cut open and the contents carefully examined. All helminths found were fixed in 70 or 95% ethanol, and identified depending on the taxonomic group following Bray and Gibson (1990) for digeneans, Berland (1961) for anisakid nematodes, Yamaguti (1971) for didymozoids, Rohde and Watson (1985) and Rohde (1989) for monogeneans and Khalil *et al.* (1994) for cestodes. Subsequently, nematodes were subjected to molecular identification according to D'Amelio *et al.* (2000) and Pontes *et al.* (2005) as follows: Genomic DNA was isolated using Wizard® Genomic DNA purification kit (Promega) and the nuclear ribosomal ITS region (plus intervening 5.8S rRNA gene) was amplified by PCR using 5.0 µl of template DNA (20–40 ng), 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub> (Bioline), 40 mM of a nucleotide mix (Promega), 50 pM/µl of each the forward primer NC5 (5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and the reverse primer NC2 (5'-TTAGTTTCTTCCTCCGCT-3') (Zhu *et al.* 2000) and 1.0 U of BIOTAQ DNA Polymerase (Bioline) in a final volume of 50 µl. The PCR was performed in a GeneAmp PCR System 2400 (Applied Biosystems) under the conditions described in following conditions: 10 min at 95°C (initial denaturation), 30 cycles of 30 sec at 95°C (denaturation), 40 sec at 52°C (annealing) and 75 sec at 72°C (extension), and a final elongation step of 7 min at 72°C. The amplicons obtain were digested with endonucleases (*HinfI* and *HhaI*), which proved to be of diagnostic value among anisakid nematodes (D'Amelio *et al.* 2000, Pontes *et al.* 2005). Prevalence and abundance of infection were calculated according to Bush *et al.* (1997). Relationships of fish length and abundance of infection were analysed by Spearman rank correlation coefficient using SPSS 12.0. Differences in prevalence of helminth parasites occurring in Canary Islands and other geographic regions within the Atlantic Ocean were studied using the  $\chi^2$  test.

A total of 11 parasite taxa were found infecting the 68 Atlantic chub mackerel, with total lengths ranging from 19 to 25 cm (21.42 ± 0.99) and total weight from 50 to 91.4 g (65.43 ± 8.79) (Table I). Three species of monogeneans were found infecting the gills. The monogenean *Pseudokuhnia minor* Rohde et Watson, 1985 was by far the most abundant species with prevalence reaching 54.4%. On the other hand, *Kuhnia scomberi* (Kuhn) Sproston, 1945 and *K. scombercolias* Nasir et Fuentes Zambrano, 1983 were only found in one fish each. The digenean *Prodistomum orientalis* Layman, 1930 was similarly found in one single fish. Eighteen L3 larval anisakid nematodes were found infecting the body cavity of 8 fish. Twelve of them yielded positive PCR amplicons of about 1000 bp. The PCR-RFLP analysis, using two diagnostic endonucleases (D'Amelio *et al.* 2000), allowed the identification of three different species of the genus *Anisakis* (*A. pegreffii* Campana Rouget et Biocca, 1955, *A. nascettii* Mattiucci, Paoletti et Webb, 2009, and *A. typica* Diesing, 1860) and one species of the genus *Pseudoterranova*, *P. ceticola* (Deardorff et Overstreet, 1981). Eight larvae from *S. colias* showed the restriction profiles of *A. pegreffii*: i.e. three fragments of about 370, 300 and 250 bp after digestion with *HinfI* and two fragments of about 550 bp and 430 bp after digestion with *HhaI*. Of the remaining three larvae from *S. colias*, two were identified as *A. typica*, showing two fragments of about 620 bp and 350 bp after restriction with *HinfI* and four fragments of about 320 bp, 240 bp, 180 bp and 160 bp using *HhaI* endonuclease. Another larva was identified as *A. nascettii*, on the basis of its restriction patterns after digestion with *HinfI* (two fragments of about 650 bp and 300 bp) and with *HhaI* (three fragments of about 550 bp, 280 bp, 120 bp), according to the restriction profile described for *Anisakis* sp. A in Pontes *et al.* (2005) and named *A. nascettii* in Mattiucci *et al.* (2009). Finally, the digestion of PCR product of another individual recovered in *S. colias* with *HinfI* produced a single undigested band at about 1000 bp while *HhaI* produced a pattern of four bands at 400, 200, 180 and 80 bp. This profile is not referable to any *Anisakis* species, but allowed the identification of this individual worm as *Pseudoterranova ceticola*, according to the restriction pattern defined for this species on adult specimens from cetaceans from the Gulf of Mexico (unpublished data). The remaining anisakids (6) could not be identified to species level. The acanthocephalan *Rhadinorhynchus pristis* (Rudolphi, 1802) Lühe, 1911, was recovered from the intestine of 3 fish. Bright yellow didymozoid digeneans were found either attached to gill filaments or viscera, free or encysted. We were unable to identify these didymozoids, due to their poor conservation status after defrosting. The only didymozoid which we could identify was *Nematobothrium scomberi* (Taschenberg, 1879) Ishii, 1935, infecting the opercula of one chub mackerel. The larval tetraphyllidean, *Scolex pleuronectis* Müller, 1788, was found infecting 6 fish. These metacestodes resembled metacestodes of type 7 of Chambers *et al.* (2000). Additionally, cysts of a bright yellow color, containing a larval cestode of the order Trypanorhyncha, were found attached



<b>Acanthocephala</b>															
<i>Bolbosoma</i> sp. (L.)	0	0	0	0	0	0	0	0	0	0	0	0			
<i>Bolbosoma vasculosum</i> (L.)	0	0	2.0	0.02	0	0	0	0	0	0	0	0			
<i>Corynosoma australe</i> (L.)	0	0	0	0	0	0	0	0	0	12.0	0.17	64.4			
<i>Corynosoma</i> sp. (L.)	0	0	0	0	0	0	0	0	0	10.0	0.21	0			
<i>Rhadinorhynchus cadenati</i>	0	0	0	0	0.04	59.9	4.43	15.8	0.20	0	0	0			
<i>R. pristis</i>	4.4	0.04	48.3	0.64	0	0	0	0	0	7.0	0.13	0			
<b>Nematoda</b>															
<i>Anisakis</i> sp. (L.)	11.8	0.25	53.6	1.01	16.66	87	5.78	55.5	2.83	11.8	0.20	4.0	0.14	36.7	0.7
<i>Camallanus</i> sp.	0	0	0	0	0	15.2	0.26	0	0	0	0	0	0	0	0
Capillariidae gen. sp.	0	0	0	0	0	0	0	0	0	1.3	0.01	0	0	0	0
<i>Contracaecum</i> sp. (L.)	0	0	0	0	24.1	2.38	4.3	5.5	0.06	3.9	0.11	2.0	0.05	26.7	0.6
<i>Hysterothylacium fabri</i> (L.)	0	0	7.3	0.12	0	0	0	0	0	0	0	0	0	0	0
<i>Hysterothylacium</i> sp. (L.)	0	0	22.5	0.58	0	0	0	22.5	0.85	19.7	0.64	0	0	50.0	1.9
<i>Oncophora melanocephala</i>	0	0	6.0	0.03	0	0	0	0	0	0	0	0	0	0	0
<i>Pseudoterranova</i> sp.(L.)	1.5	0.01	0	0	0	0	0	0	0	0	0	0	0	6.7	0.10
<i>Raphidascaris</i> sp. (L.)	0	0	0	0	0	0	0	0	0	0	0	52.0	14.25	0	0
<b>Copepoda</b>															
<i>Caligus pelamydis</i>	0	0	0	0	0	0	0	0	0	0	0	2.0	0.02	0	0
<i>Clavellisa scombri</i>	0	0	7.9	0.12	0	0	0	0	0	0	0	2.0	0.02	0	0

to the external walls of the stomach of 6 fish. Hook patterns suggest that the larvae belong to a trypanorhynchean with a heteroacanthous pattern of tentacular armature. A total of 6 of these cysts were found. Table I gives the results of prevalence and abundance of the different parasite species found infecting the Atlantic chub mackerel in the present work, as well as records of parasites found in the same fish species, obtained from a literature review. No significant relationship was found between fish length and abundance of infection for the most common parasites, namely *P. minor* ( $r = 0.210$ ,  $p = 0.086$ ); anisakid nematodes ( $r = 0.142$ ,  $p = 0.247$ ); gill didymozoids ( $r = 0.026$ ,  $p = 0.831$ ); *S. pleuronectis* ( $r = 0.153$ ,  $p = 0.212$ ).

Previous morphological and genetic studies done on *Scomber colias* from both sides of the Atlantic, indicated that differences existed between northwestern and southwestern samples, in contrast to no significant differences between the eastern Mediterranean, North Atlantic, Ivory Coast and South Africa (Scoles *et al.* 1998, Zardoya *et al.* 2004). The work of Oliva *et al.* (2008), which analysed the helminth parasites of *S. colias* and *S. japonicus* (designated as *S. japonicus* by authors) from localities within the Atlantic and southern Pacific waters, identified some parasites specific to the Atlantic and others specific to the Pacific, suggesting that indeed two different *Scomber* species inhabit the Atlantic and the Pacific oceans. Within the Atlantic, could the species represent one or more population units? Chub mackerels from Canary and Madeira Islands share the following helminth parasites: *Pseudokuhnia minor*, the most abundant monogenean species, *K. scombri*, *K. scombercolias*, *Nematobothrium scombri*, *Prodistomum orientalis*, *Scolex pleuronectis*, anisakid nematodes, specifically *A. pegreffii*, *A. typica* and *A. nascettii* and the acanthocephalan *Rhadinorhynchus pristis* (Pontes *et al.* 2005, Oliva *et al.* 2008, present results). The lower values of prevalence of the helminth parasites in Canary Islands compared with those found in other regions of the North East Atlantic could be due to the difference in size of examined fish (Table I). Nevertheless different values of prevalence with some helminth parasites were also found between Madeira Island and other regions of Central North-East Atlantic, which appeared to be independent of fish size (Table I). Significant differences in prevalence with *Pseudokuhnia minor* were observed, with lower values in the Azores banks (17.2 and 17.4%) in comparison with Canary Islands (54.4%), Madeira Islands (98.7%) and Atlantic coast of Northern Africa (78% and 94.7%) (Tables I and II). Furthermore this monogenean appears to be common in subtropical North- and Southeastern coasts of the Atlantic (Shukhgalter 2004, Costa *et al.* 2007), but not in the Southwest Atlantic (Rohde and Watson 1985, Cremonte and Sardella 1997, Alves *et al.* 2003, Oliva *et al.* 2008). Other monogeneans, e.g. *Kuhnia scombri* and *K. scombercolias* showed also significant differences in the prevalence values depending on sampling region (see Table I). Prevalence with *Nematobothrium* species showed identically differences between regions, with lower prevalence in Canary Islands (7.4%) and Azores (2.2%), against higher prevalence in

**Table II.** Significance of prevalence differences (frequency of infected fish) of some helminth parasites of the Atlantic chub mackerel, *S. colias*, in different geographic regions, using the chi-square test

Parasite species	Can/Mad	Can/Mor	Can/Mau	Can/Az	Mad/Mor	Mad/Mau	Mad/Az	Az/Mor	Az/Mau	Mau/Mor
<i>Pseudokuhnia minor</i>	71.78 p = 0.000	13.57 p = 0.000	31.72 p = 0.000	11.44 p = 0.001	31.94 p = 0.000	3.05 p = 0.08	130.55 p = 0.000	43.73 p = 0.000	64.46 p = 0.000	10.59 p = 0.001
<i>Kuhnia scomбри</i>	39.39 p = 0.000	12.67 p = 0.000	1.13 p = 0.29	13.80 p = 0.000	23.4 p = 0.000	46.84 p = 0.000	3.87 p = 0.05	0.38 p = 0.54	19.66 p = 0.000	16.91 p = 0.000
<i>Nematobothrium</i> sp.	9.88 p = 0.002	16.45 p = 0.000	7.00 p = 0.008	2.73 p = 0.099	57.93 p = 0.000	25.94 p = 0.000	10.52 p = 0.001	–	–	–
<i>Contracaecum</i> sp.	–	3.89 p = 0.05	2.74 p = 0.10	17.69 p = 0.000	8.55 p = 0.003	6.04 p = 0.01	37.92 p = 0.000	11.74 p = 0.001	9.93 p = 0.002	0.27 p = 0.61
<i>Rhadinorhynchus</i> sp.	39.94 p = 0.000	61.61 p = 0.000	4.98 p = 0.03	43.29 p = 0.000	4.44 p = 0.04	22.87 p = 0.000	2.87 p = 0.09	0.00 p = 0.57	24.95 p = 0.000	41.87 p = 0.000
<i>Hysterothylacium</i> sp.	25.51 p = 0.000	18.32 p = 0.000	14.98 p = 0.000	–	2.28 p = 0.13	2.63 p = 0.11	11.52 p = 0.001	8.11 p = 0.004	6.68 p = 0.01	0.246 p = 0.62

Can – Canary Islands, Mad – Madeira Islands, Az – Azores banks, Mau – Mauritania, Mor – Morocco; p – level of significance.

Madeira Islands (27.8%) (Tables I and II). It was absent from Atlantic coasts of Morocco and Mauritania (Shukhgalter 2004, Oliva *et al.* 2008). The digenean, *Lecithocladium excisum* occurred only in Morocco and Mauritania (41.2 and 55.3%, respectively) and the Azores banks (2.2%). Regarding infections with larval cestodes, it was particularly noted that *Nybelinia* sp. was exclusive of Madeira and Azores Islands (Table I), whereas in other regions of the North Atlantic the cestode fauna was represented by other species. The acanthocephalan *Rhadinorhynchus pristis* was common in Central North-East Atlantic, also with significant differences in prevalence, but absent from the Argentinean coastal waters (Cremonte and Sardella 1997, Oliva *et al.* 2008). As the identification of *Anisakis* larvae to species level was only done for the specimens collected from the Canary Islands (present results) and Madeira (Pontes *et al.* 2005), the values of prevalence given in Table I, are representative of all individuals of the genus *Anisakis*. Significant differences were observed in the prevalence of *Anisakis* sp. between regions. From the three species identified by molecular markers in the present work, *Anisakis pegreffii* is common throughout the Mediterranean, having been found at Madeira (Pontes *et al.* 2005), Morocco and Mauritania (Mattiucci *et al.* 1997; Farjallah *et al.* 2008), extending its distribution to the southern Atlantic (Mattiucci and Nascetti 2008). This anisakid together with *A. typica*, also found in the present work, are parasites of dolphins, confined to surface waters and from 35°N to the austral region (see Mattiucci *et al.* 1997). On the other hand, the recently named *A. nascettii*, (Mattiucci *et al.* 2009), first reported from Madeira as *Anisakis* sp. A (Pontes *et al.* 2005), is typical of beaked whales, with the larvae infecting fish species from surface and deep waters (Mattiucci *et al.* 2009, Pontes *et al.* 2005). It has a wide distributional range in the Atlantic and the Pacific, where adults were found in *Mesoplodon grayi* (Mattiucci *et al.* 2009). Juveniles were also found in *M. bowdoini* and other *Mesoplodon* species in South Africa and Spanish North Atlantic waters (Iglesias *et al.* 2008). *Pseudoterranova ceticola* (Deardoff et Overstreet, 1981) a parasite of sperm whales, namely of *Kogia simus* and *Kogia breviceps*, was previously reported from the Caribbean region (Colón-Llavina *et al.* 2009) and from Mexico (González Solíz *et al.* 2006), as well as from Galician Atlantic waters (Abollo and Pascual 2002). Larvae of another anisakid, *Contracaecum* sp. reported from the Azores banks, Morocco and Mauritania did not infect *S. colias* from either Canary or Madeira Islands (Shukhgalter 2004, Oliva *et al.* 2008, present results). Larval *Hysterothylacium* sp. showed higher prevalence in Madeira (22.5%), Morocco (22.5%) and Mauritania (19.7%) been absent from Canary Islands and Azores banks (Tables I and II). Although we found common parasite species to all the regions of the Central North Atlantic (presence of *Anisakis* sp., *Pseudokuhnia minor*, *K. scomбри* and *Rhadinorhynchus* species), which may suggest a single population of *S. colias*, there are some differences that may need further investigations (see Table II). In particular, the absence of *Lecithocladium excisum* and *Con-*

*tracaecum* sp. from Madeira and Canary Islands and some of the Azores banks, the occurrence of the acanthocephalan *Bolbosoma vasculosum* in the Azores and Madeira Islands only (Shukhgalter 2004, Oliva *et al.* 2008), as well as the restriction of the trypanorhynch *Tentacularia coryphaenae* to Madeira (Oliva *et al.* 2008, present results). On the southwestern side of the Atlantic, the presence of the anisakid *Raphidascaris* sp., the acanthocephalan *Corynosoma australe*, the digenean *Lecithocladium harpodontis* and the absence of *Pseudokuhnia minor* might indicate another population unit (Cremonte and Sardella 1997, Oliva *et al.* 2008).

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