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VERSITA

Endohelminth parasites of the leafscale gulper shark, *Centrophorus squamosus* (Bonnaterre, 1788) (Squaliformes:Centrophoridae) off Madeira Archipelago

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Abstract

The endohelminth parasite fauna of a deep water shark, the leafscale gulper shark, *Centrophorus squamosus*, examined from Madeiran waters, from September 2009 to January 2010, consisted of larval and juvenile cestodes of two orders, namely Trypanorhyncha and Tetraphyllidea, and L3 stages of *Anisakis* spp. Infection with *Anisakis* spp. could be due to the shark's opportunistic feeding on squids and black-scabbard fish, *Aphanopus carbo*, which is heavily parasitized by *Anisakis* spp. in Madeira waters. The occurrence of larval and juvenile cestodes only, in this shark, suggests that the leafscale gulper shark features as a paratenic or a dead-end host for the parasites.

Keywords

Tetraphyllidea, Trypanorhyncha, Anisakidae, *Centrophorus squamosus*, Madeira Island

Introduction

The leafscale gulper shark, *Centrophorus squamosus* (Bonnaterre, 1788) (Squaliformes: Centrophoridae) is a bathydemersal marine shark species, occurring in the eastern Atlantic from Iceland to South Africa, in the western Indian Ocean and in the western Pacific Ocean (Compagno 1984, Froese and Pauly 2011). This species is commercially exploited and its flesh is used for human consumption and for fishmeal, whereas the liver is used for oil extraction. Some aspects of the biology of *C. squamosus*, namely age and growth and reproductive strategies were investigated to date by several authors (Girard and Du Buit 1999, Clark *et al*. 2001, Bañon *et al*. 2006, Figueiredo *et al*. 2008, White and Dharmadi 2010). In Madeira Archipelago, this shark species is caught with the deep-water long line used for the black scabbard fishing, with reported landings for the period 2008–2011 of 787.6 tons (Direcção Regional das Pescas, Funchal, unpublish.).

The order Squaliformes includes 115 species, distributed in seven families, found mainly in deep-waters (Compagno *et al*. 2005). Fifteen species are assigned to the family Centrophoridae. Nevertheless, the taxonomy and the ecology of *Centrophorus* and other squaliform genera, is still poorly known and the genus is undergoing taxonomic revisions (Guallart *et al*. 2006, Moura *et al*. 2008, White and Dharmadi 2010). Parasites can be interesting as indicators of elasmobranch biology, namely feeding ecology, and they may help in the identification of the host, if they are strictly host specific (Caira 1990). Records of parasites occurring in *C. squamosus* as well as in other species of deep-water sharks are rare, probably because of the difficulties of fishing in the deep waters. A few surveys done by Campbell (1990) and Klimpel *et al*. (2003) suggested that the helminth faunas of deep water sharks are less rich in species than those of pelagic sharks. Nevertheless, more surveys were done to study the parasites of pelagic shark species in comparison with those done for deep water species, so that a great lack of information still exists concerning the parasites of deep water sharks. In particular, the only records of the occurrence of parasites in the leafscale gulper shark elsewhere, are those of plerocerci of the trypanorhynchs *Grillotia erinaceus* (van Beneden, 1858), *G. dolichocephala* (Guiart, 1935) (syn. *G. minor*) (Lacistorynchidae) (Beveridge et Campbell, 2013) and adults of *Chimaerarhynchus rougetae* (Beveridge et Campbell, 1989) (Gymnorhynchidae) described from *Centrophorus* sp.

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The main objective of the present study was to conduct an extended survey of the endohelminth parasites infecting sharks of the genus *Centrophorus* commercially exploited in Madeira Archipelago, in order to add to the scarce information on the occurrence of parasites of deepwater sharks.

Materials and Methods

Collection of shark samples

A total of sixty-nine individuals of *Centrophorus squamosus* caught at Madeira Archipelago (Northeast Atlantic) (32°22´20˝N and 16°16´30˝N) were examined from September 2009 to January 2010. Sharks were obtained from a commercial fish processing industry near Funchal, Madeira Island (SOPEIXE). Fifty-seven sharks were measured in centimetres (cm) and sexed. The stomachs and spiral intestines of all sharks were collected in individual plastic bags.

Morphological examination of parasites

At the laboratory stomachs and spiral intestines were dissected and all the endohelminths recovered were placed in petri-dishes with sea water. Cestodes were fixed in 4% formalin buffered with sea-water or 70% ethanol, and nematodes were fixed in 70% ethanol. Four scolices of the ethanol preserved cestodes, were hydrated in descending series of ethanol to distilled water, post-fixed with 2% osmium tetroxide buffered in sodium cacodylate overnight, dehydrated in ascending series of ethanol, critical point dried with a Baltec CPD 030 and examined with a FEI QUANTA 400 FEG ESEM. The remaining cestodes were examined by light microscopy, either, unstained, mounted in glycerine, or, stained with acetic carmine, dehydrated in ascending ethanol series, cleared in eugenol and mounted with entellan. Anterior portion of the nematodes fixed in 70% ethanol were cleared in lactophenol and examined by light microscopy. Remaining of their bodies was kept for molecular analysis. Identification of nematodes and cestodes followed Berland (1961) and Palm (2004).

Molecular analysis of nematodes

Genomic DNA of 8 nematodes 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 MgCl2 (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'-TTAGTTTCTTCCTC-CGCT-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 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 obtained were digested with endonucleases (*HinfI*, *HhaI*), which proved to be of diagnostic value among anisakid nematodes (D'Amelio *et al*. 2000, Pontes *et al.* 2005).

Host Infections

Infections with endohelminths were evaluated by calculating the parasitological descriptors, prevalence (P) mean intensity (MI) and mean abundance (MA) according to Bush *et al.* (1997). Measurement units are centimetres (cm) for shark length, and millimeters (mm) and micrometers (µm) for parasites.

Results

Host Length Data

Fifty-seven individuals of *C. squamosus* examined from September 2009 to January 2010, ranged in length from 107 to 136 cm (111.4 ± 5.4; mean ± std, n = 57) (Fig. 1). Males predominated in the samples (n = 54). The remaining sharks (n = 12) were not measured and sexed.

Host Infections

The endohelminth fauna of *C. squamosus* consisted of larval or juvenile cestodes of two orders, Tetraphyllidea and Trypanorhyncha, and larval nematodes of the family Anisakidae. No adult cestodes or nematodes were found in the 69 sharks examined (Table I). Thirteen sharks were infected with larval trypanorhynchs (P = 18.84%, n = 69), either as white oval cysts, measuring 1 to 3 mm long or as plerocerci, found in the stomach. Two of these sharks were infected with plerocerci of *Tentacularia coryphaenae* Bosc, 1797 (P = 2.90%), eight with cysts containing larvae of cestodes apparently belonging to the family Gilquiniidae Dollfus, 1942 (P = 11.60%), and 3 sharks were infected with unindentified cestode cysts (P = 4.35%). Nine sharks were infected with juvenile triloculate tetraphyllideans (P = 13.04%).

Morphological examination of parasites

Trypanorhyncha

A total of 71 white oval cysts measuring 1 to 3 mm long, and 3 plerocerci of trypanorhynchs were found infecting the stomach and spiral intestine of 11 *C. squamosus.* Sixty-six of those cysts showed an evaginated scolex with four oval shaped bothria and invaginated tentacles armed with heteromorphous hooks, which suggested they belonged to the family Gilquiniidae (Dollfus,

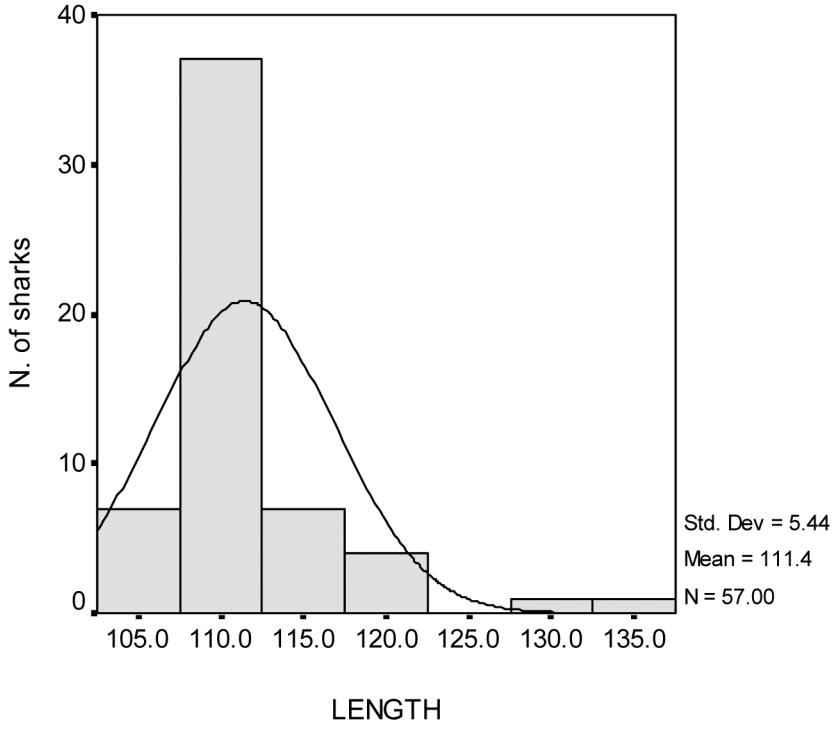

Fig. 1. Length frequency distribution of *Centrophorus squamosus*

1942). The remaining 5 cysts could not be assigned to any family. Two plerocerci of *Tentacularia coryphaenae* (Bosc, 1797) were found in the stomach of 2 *C. squamosus*.

Tetraphyllideans

Nineteen juvenile specimens of cestodes belonging to the order Tetraphyllidea were found, 18 in the stomach and one in the spiral intestine of *C. squamosus*. The scolex of these specimens was adorned with 4 oval shaped sessile muscular bothridia, each with 3 loculi, without hooks or suckers (Fig. 2). The apex of the scolex had no sucker and was dome shaped (Fig. 2). The strobila of these worms was acraspedote. They had a mean length of 16.2 mm (10–23 mm, n = 6). The bothridia, with 3 loculi, were 567 to 768 µm long and 329 µm wide, and the loculi were 122–183 µm long and 146–183 µm

Table I. Prevalence and mean intensity (± S.E.) values of the endohelminths found infecting the lumen of the stomach and spiral intestine of the 69 leafscale gulper sharks, *Centrophorus squamosus*, from Madeiran waters, from September 2009 to January 2010

Parasite species	N	Prevalence (%)	Mean intensity (range)	Site of infection
Trypanorhyncha				
Gilquiniidae (L)	66	11.6	8.25 ± 2.25 (1–19)	Stomach, spiral intestine
Unidentified (L)	5	4.35	1.67 ± 0.67 (1–3)	stomach
Tentacularia coryphaenae (L)	2	2.90	1	stomach
Tetraphyllidea				
Triloculate type (juveniles)	19	13.04	2.11 ± 0.35 (1–4)	Stomach, spiral intestine
Nematoda				
Anisakis simplex (L3)	7	5.80	1.5 ± 0.29 (1–2)	stomach
Pseudoterranova ceticola	1	1.45	1	stomach
Unidentified	1	1.45	–	stomach

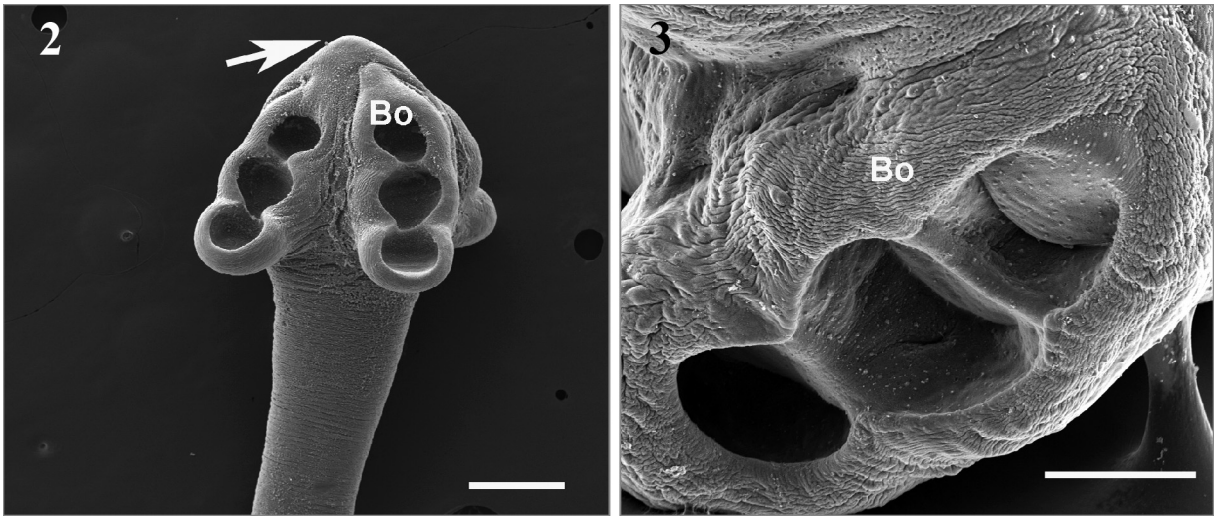

Fig. 2. Scolex of one tetraphyllidean juvenile showing the triloculate type of bothridia (Bo) and pointed-shaped apex (arrowed). Bar = 300 µm. **Fig. 3.** Detail of the bothridia (Bo) of a triloculate juvenile of tetraphyllidean. Bar = 100 µm

wide (Fig. 3). Some of these triloculate juveniles had a flattened scolex instead of the dome shaped one. Voucher specimens have been deposited in the collection of the Natural History Museum of Funchal, Marine Biological Station, with the number MMF nº 43030.

Nematodes

The nine larval nematodes found in the stomach of *C. squamosus* were morphologically identified as anisakids. Eight of these larvae were further subjected to molecular identification. Based on the results of the PCR-RFLPs analysis, seven larvae corresponded to *Anisakis simplex s.s.* (Rudolphi, 1809), and one to *Pseudoterranova ceticola* (Deardorff et Overstreet, 1981). The seven larvae identified as *A. simplex s.s*. were characterized by the following restriction profiles: two fragments (550, 430 bp) with *HhaI* and three fragments (620, 250, 80 bp) with *HinfI*. The restriction profile of *Pseudoterranova ceticola* with *HhaI* was composed of 4 fragments (400, 200, 180, 80 bp) and with *HinfI* a single undigested band of about 1000 bp.

Discussion

Adults and plerocerci of trypanorhynchs have been reported from sharks of the family Centrophoridae: (1) from *Centrophorus granulosus* (Bloch et Schneider, 1801) adults and plerocerci of *Sphyriocephalus viridis* (Wagener, 1854) Pintner, 1913, plerocerci of *Grillotia scolecina* (Rudolphi, 1819) regarded as a *species inquirenda* according to Beveridge and Campbell (2013), and *Grillotia* sp.; (2) from *C. moluccensis* (Bleeker, 1860), adults of *Gilquinia robertsoni* (Beveridge, 1990), and plerocerci of *Tentacularia coryphaenae* (Bosc, 1797); (3) from *Centrophorus squamosus* (Bonnaterre, 1788), plerocerci of *Grillotia erinaceus* (van Beneden, 1858) and *G. dolichocephala* (Guiart, 1935) (syn. *G. minor*); (4) from *Centrophorus* sp., adults of *Chimaerarhynchus rougetae* (Beveridge and Campbell, 1989), *Gilquinia minor* (Beveridge *et* Justine, 2006) and *Sagittirynchus aculeatus* (Beveridge et Justine, 2006) (see Palm 2004, Beveridge and Justine 2006). These results give support to our observations, suggesting that the plerocerci, and probably the cysts of trypanorhynchs, found in the present study belong to the family Gilquiniidae (Dollfus, 1942), a small family of cestodes with six described genera (Palm 2004, Beveridge and Justine 2006). Cestodes of this family occur predominately in deepwater squalid sharks (Klimpel *et al*. 2001).

In contrast to the variety of species of trypanorhynchs infecting deepwater sharks of the genus *Centrophorus*, a single tetraphyllidean cestode was found to date in these sharks by Oberstein (1913 *in* Euzet 1994). This author reported the occurrence of a tetraphyllidean, *Bilocularia hyperapolytica* in *C. granulosus* from North Atlantic, which Euzet (1959) renamed as *Calyptrobothrium rigii* (Monticelli, 1893). Apart from this record no further records of the occurrence of tetraphyllideans were found for sharks of the genus *Centrophorus*. Alexander (1963), referred to the occurrence of *Monorygma hyperapolytica* (Obersteiner, 1913) in the deep-water shark, *Dalatias licha*, off New Zealand and recently Tazerouti *et al.* (2007) considered that *B. hyperapolytica* (found in *Centrophorus granulosus*) resembles the phyllobothridian *Calyptrobothrium riggii* (Monticelli, 1893). *Centrophorus granulosus* lives in the same habitat of *C. squamosus* and is also caught in Madeira but in much less quantity. Because they are both commercially exploited and have low reproduction potential, catches have been declining, so that both species are listed as vulnerable in the IUCN database (White 2003, Gualart *et al*. 2006). They both feed on fish and squids and occupy the same trophic level (4.2) (Cortés 1999). Squids and several teleost species act as intermediate/paratenic hosts for many trypanorhynchs of the gen-

era *Grillotia* and *Gilquinia* (Palm 2004). Another squalid shark species, which perhaps due to overexploitation disappeared from the catches in Madeira, *Centroscymnus coelolepis* Barbosa du Bocage and Brito Capello, 1864, occupies also the same trophic level (4.2) but feeding on squids is much more important (79.0 against 12.0 and 34.0 for *C. granulosus* and *C. squamosus* respectively). Besides it feeds also on other sharks. Could this species be the true definitive host of the juvenile and larval cestodes found in this study? Other sharks with similar feeding habits, including squid and fish, as well as sharks, known to exist in the area but not reported in catches, such as *Hexanchus griseus* (Bonnaterre, 1788), and *Squalus acanthias* (Linnaeus, 1758) (Froese and Pauly 2011) could be alternatives for definitive hosts of the cestodes found in the present study.

The occurrence of juvenile tetraphyllideans in the stomach of sharks is not unusual and was previously reported for *Trilocularia acanthiaevulgaris* (family Triloculariidae Yamaguti, 1959) found in *Squalus acanthias* (see McCullough and Fairweather 1983). *T. acanthiaevulgaris* has two forms of bothridia, while in the stomach they are ovoid shaped, contrariwise in the spiral intestine they are more triangular in shape (McCullough and Fairweather 1984). Furthermore this species releases the proglottides, of 1.0–1.5 mm in length, which are found detached in the spiral intestine (McCullough *et al.* 1986). This phenomenon of detachment of immature proglottides, known as hyperapolysis, is common to many tetraphyllidean species (Euzet 1959). Could our immature worms have a similar development? Mature proglottides were not found in the present study so that the true identity of the tetraphyllidean found cannot be determined at this point. It appears that it might belong to the family Triloculariidae (Berman and Brooks 1994).

The fact that only larval stages of trypanorhynchs and juveniles of tetraphyllideans were found in *C. squamosus* in the present study further suggests that this shark is not the definitive host, but either a paratenic or a dead-end host.

Although the occurrence of larval anisakid nematodes is rare in sharks, there have been occasional reports of their occurrence in elasmobranchs (Palm and Schröder 2001, Klimpel *et al.* 2003). Adults of nematodes of the genus *Anisakis* are parasites of marine mammals, whereas L3 larvae infect a while variety of teleost fish and squids (Mattiucci *et al.* 1997, Mattiucci and Nascetti 2008). Their occurrence in *C. squamosus*, in the present study, appears to be correlated to the consumption of squids and fish. In particular, diet of this shark in Madeiran waters, showed a predominance of deep-water squids, namely *Chiroteuthis* sp., *Vampyroteuthis* sp. and Onycoteuthidae (Freitas 1998) also common prey items of the black scabbard fish, *Aphanopus carbo* (Lowe, 1839) (Freitas 1998) a deep-water fish, with high anisakid nematode burdens (Costa *et al.* 2003). Additionally the shark also feeds on *A. carbo*. However, the low prevalence of anisakids found (5.8%) in comparison with high prevalence found in the deep-water fish *A. carbo* (up to 100% see Costa *et al.* 2003) occupying the same habitat, may suggest that *A. carbo* represents an adequate paratenic host, as many other fish species are, while *C. squamosus*, where the larvae are unusually found in the stomach, represent an accidental, unsuitable host for anisakid larvae. Molecular analysis of a sample of larval anisakids from *C. squamosus*, identified two species, *Anisakis simplex s.s.* and *Pseudoterranova ceticola*. The former is a widespread anisakid species in the northern hemisphere, and it has been already detected in Madeiran waters in three fish species, *A. carbo*, *Trachurus picturatus* and *Scomber japonicus* (Pontes *et al.* 2005), while the latter is commonly found in warm waters, e.g. Gulf of Mexico (Cavallero *et al.* 2011) and is likely due to predation on squids.

Conclusions

In conclusion, *C. squamosus* appears to play a role as intermediate/paratenic host for cestodes, both trypanorhynchs and tetraphyllideans, and as accidental host in regard to anisakid nematodes. Moreover, the larval trypanorhynchs found in the stomach and spiral intestine of this shark are generalist species, with adult forms parasitizing more than one shark species (see Palm 2004), whereas the tetraphyllidean juveniles and larvae could be members of the family Triloculariidae (Yamaguti, 1959), characterized by the possession of scolex with four bothridia divided by septa and the absence of myzorhynchus. The fact that in a top predatory shark no single adult of a tetraphyllidean was found, may indicate that overexploitation of the suitable definitive hosts, are leading the parasites to a dead end. In reality, many of the deepwater sharks present in North Atlantic have been classified as vulnerable (*C. squamosus*, *C. granulosus*) and near threatened (*Centroscymnus coelolepis* and *Hexanchus griseus*). In Madeira, catches of *C. squamosus* showed fluctuactions from 1990 up to present date, however the amount fished in the period 2008–2011 was high (787.6 tons). On the contrary *C. coelolepis* disappeared from catches since 1997 up to 2008 and in the period 2008–2011 only 340 kg were caught. *C. granulosus*, *H. griseus* and *S. acanthias* are also rare and not reported from the commercial fisheries in Madeira.

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