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

Graça Costa¹,²*, Tomás Chada¹, Egberto Melo-Moreira¹, Serena Cavallero³ and Stefano D’Amelio³

¹Estação de Biologia Marinha do Funchal, Universidade da Madeira, Promenade Orla Marítima do Funchal, 9000-107 Funchal, Portugal; ²ISOPlexis, Universidade da Madeira, Campus da Penteada, 9000-390 Funchal, Portugal; ³Department of Public Health Sciences, University of Rome La Sapienza, Piazzale Aldo Moro, 00185 Italy

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 Klímpel 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 plecrocerci 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.

*Corresponding author: gcosta@uma.pt*
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′-GTAGGTTGAACCTGCGGAAGGATCATATT-3′) and the reverse primer NC2 (5′-TGATTTTCTTCCTC-GGT-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 unidentified 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,