International Journal of Food Microbiology 235 (2016) 113–124

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Integrative taxonomy of Anisakidae and Raphidascarididae (Nematoda) in *Paralichthys patagonicus* and *Xystreurys rasile* (Pisces: Teleostei) from Brazil

Michelle Cristie Gonçalves da Fonseca [a], Marcelo Knoff [b,*], Nilza Nunes Felizardo [a], Maria Isabel N. Di Azevedo [c], Eduardo José Lopes Torres [d], Delir Corrêa Gomes [b], Alena Mayo Iñiguez [c], Sérgio Carmona de São Clemente [a]

[a] Laboratório de Inspeção e Tecnologia do Pescado, Faculdade de Medicina Veterinária, Universidade Federal Fluminense, Rua Vital Brazil Filho, 64, Vital Brazil, Niterói, RJ 24230-340, Brazil
[b] Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil, 4365, Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil
[c] LABTRIP, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil, 4365, Manguinhos, Rio de Janeiro, RJ 21045-900, Brazil
[d] Laboratório de Helmintologia Romero Lascasas Porto, Departamento de Microbiologia, Imunologia e Parasitologia, Escola de Ciências Médicas, Universidade Estadual do Rio de Janeiro, Rua Prof. Manuel de Abreu 444, 5° andar, Vila Isabel, Rio de Janeiro, RJ 20550-170, Brazil

ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 4 July 2016
Accepted 20 July 2016
Available online 22 July 2016

Keywords:
Anisakidae
Raphidascarididae
Nematodes
Paralichthys patagonicus
Xystreurys rasile
Brazil

ABSTRACT

Thirty-six *Paralichthys patagonicus* and 30 *Xystreurys rasile* were collected in the state of Rio de Janeiro, Brazil to investigate the presence of anisakid and raphidascaridid nematodes. *Anisakis typica*, *Terranova* sp., *Contracaecum* sp., *Hysterothylacium deardorffoverstreetorum*, and *Raphidascaris* sp. were identified using integrative taxonomy of morphological and genetic data. Morphological and morphometric analysis was conducted using bright field microscopy with scanning electron microscopy for topographic characterization of the cuticular surface. Phylogenetic analysis, using ITS and *cox*2 molecular targets, clearly demonstrated the species identification of *A. typica* and *H. deardorffoverstreetorum* and the high diversity of *H. deardorffoverstreetorum*. This is the first report of *A. typica*, *H. deardorffoverstreetorum*, and *Raphidascaris* sp. parasitizing *P. patagonicus* and *X. rasile*.

1. Introduction

Flounder of the family Paralichthyidae represent a primary fishery resource in the coastal waters of Brazil (Figueiredo and Menezes, 2000) and are widely sold in domestic and foreign markets.

Parasitic nematodes are important pathogens associated with human and animal health. Some inhabit the marine environment, where they are widespread in a variety of hosts. Adults are commonly found in the digestive tract of marine mammals. Larvae infect aquatic invertebrates and non-mammalian vertebrates as intermediate hosts (Klimpel and Palm, 2011). In Brazil, anisakid and raphidascaridid nematodes have been reported to parasitize some species of marine teleost fish (Fontenelle et al., 2013, 2015; Knoff et al., 2007, 2013; Ribeiro et al., 2014).

Helminth parasites have been reported in flounder of Paralichthyidae in South America, including Brazil. (Alarcos and Timi, 2012; Alarcos et al., 2016; Felizardo et al., 2009a, 2009b, 2010, 2011; Fonseca et al., 2012; Knoff et al., 2012).

Molecular investigation is an important tool for species identification, in the taxonomic studies of larvae and adult anisakid and raphidascaridid nematodes, and determining their geographical distribution (Di Azevedo et al., 2015; Knoff et al., 2012; Kong et al., 2015; Shamsi et al., 2013).

The aim of the present study was to investigate the presence of the anisakid and raphidascaridid nematode larvae in *P. patagonicus* and *X. rasile* from the State of Rio de Janeiro, Brazil. The nematode species were identified by genetic and morphological characters, and indices of prevalence, mean intensity, mean abundance, range of infection, and infection sites were determined.

2. Materials and methods

2.1. Hosts, study area, and parasite collection

Thirty-six *Paralichthys patagonicus*, mean length 40.8 cm (28.5–59 cm) and mean weight 820.4 g (280–2530 g), and 30 *Xystreurys rasile*, mean length 24.3 cm (11.5–31 cm) and mean weight 158.5 g (20–240 g) were obtained in small markets selling only fish caught offshore of Cabo Frio, Niterói, and Rio de Janeiro in Rio de Janeiro State, Brazil, considered the Southeastern Brazil marine ecoregion (Spalding et al., 2007). Fish

* Corresponding author.
E-mail address: knoffm@ioc.fiocruz.br (M. Knoff).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.026
0168-1605/

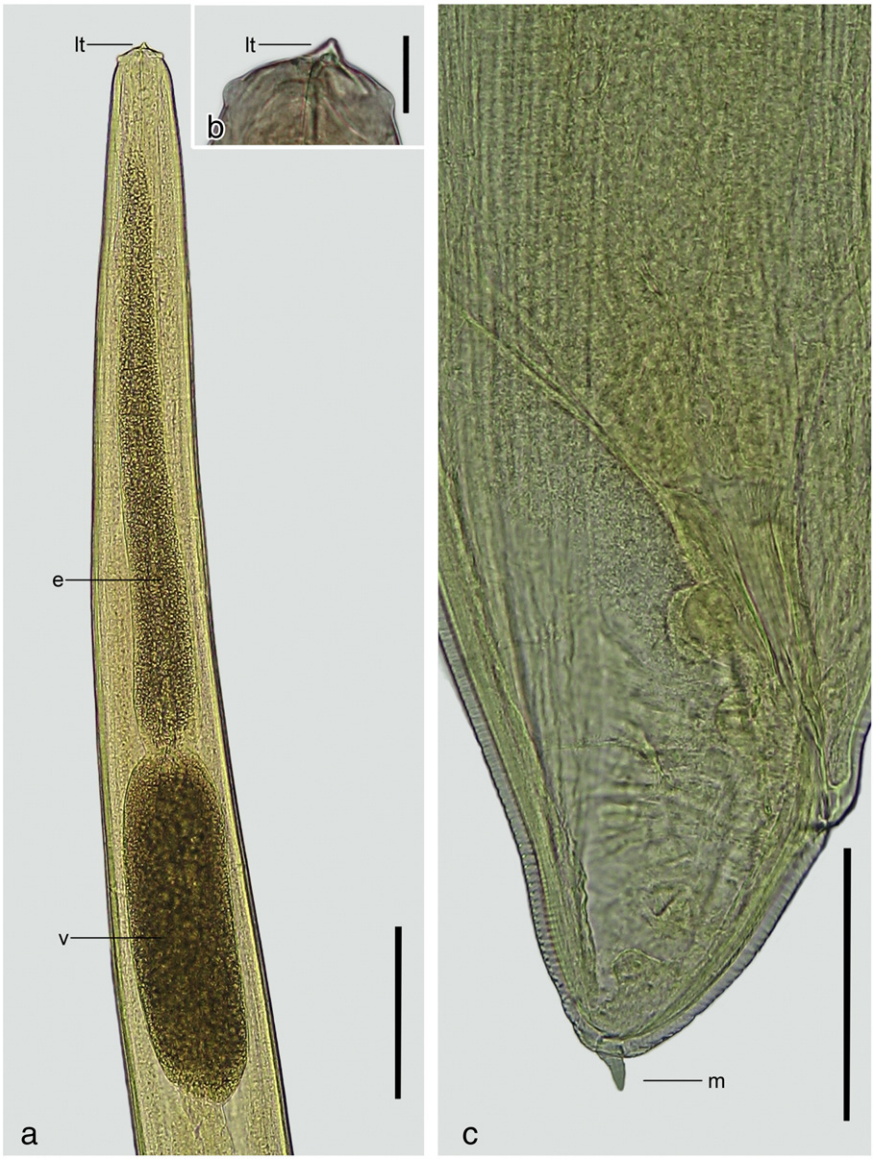

Fig. 1. *Anisakis typica* collected from *Paralichthys patagonicus*. a) anterior region, ventral view, showing larval tooth (lt), esophagus (e), and ventriculus (v); b) detail of larval tooth (lt), ventral view; c) detail of the tail showing mucron (m). Scale bars: a = 0.4 mm, b = 0.05 mm, and c = 0.2 mm.

were transported on ice to the Laboratory of Helminth Parasites of Vertebrates, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro. Species were identified according to Nakamura et al. (1986) and Figueiredo and Menezes (2000). Internal organs and musculature were examined, and nematode larvae found were placed in Petri dishes with 0.65% saline. Nematodes were fixed in AFA (ethanol, formalin, and acetic acid) at 60 °C and preserved in 70% ethanol and later clarified with Amman's lactophenol (Knoff and Gomes, 2012).

2.2. Morphological identification of parasites

Taxonomic classification was according to De Ley and Blaxter (2004), and larval identification according to Felizardo et al. (2009a) and Knoff et al. (2012). Measurements (mm) were obtained by light microscopy (Olympus BX). For topographic characterization of the cuticular surface, L_3 were examined by scanning electron microscope (SEM). The material was processed as described by Lopes Torres et al. (2013). The samples were fixed in Karnovsky solution and dehydrated in an ethanol series (70%–100%), CO_2 critical-point dried, coated in gold, examined, and photographed using SEM (JEOL SM-25 SII) under 15 kV acceleration voltage. Voucher specimens were preserved in ethanol 70° GL and deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), FIOCRUZ, Rio de Janeiro, RJ, Brazil.

Table 1
Morphometric data of *Anisakis typica* L_3 larvae collected from *Paralichthys patagonicus* and *Xystreurys rasile* in the state of Rio de Janeiro, Brazil.

A. typica	*P. patagonicus*	*X. rasile*
	$n = 5$	$n = 7$
Length	22.40–24.95 (23.91)	21.05–24.97 (23.33)
Total width	0.40–0.42 (0.41)	0.40–0.47 (0.42)
Nerve ring[a]	0.20–0.25 (0.23)	0.28–0.32 (0.29)
Esophagus (L)	1.75–1.85 (1.80)	1.50–1.70 (1.60)
Esophagus (W)	0.12–0.27 (0.17)	0.18–0.20 (0.18)
Ventriculus (L)	0.67–0.82 (0.73)	0.89–0.94 (0.79)
Ventriculus (W)	0.15–0.27 (0.18)	0.25–0.30 (0.28)
Tail (L)	0.08–0.12 (0.10)	0.09–0.13 (0.10)
Tail (W)	0.06–0.18 (0.11)	0.10–0.18 (0.14)
Mucron (L)	0.005–0.020 (0.013)	0.005–0.010 (0.007)

Measurements are in millimeters, means in parentheses. L = length; W = width; n = number of larvae measured.
[a] Distance to the anterior end.