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ANIMAL SCIENCE

Contracaecum jorgei in common snooks in Brazil: new host, locality record and remarks on misidentifications of *Contracaecum* spp. in GenBank

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Abstract: *Contracaecum jorgei* was recently described as a new anisakid species from the Argentine wolffish *Hoplias argentinensis*. This study aimed to report a new host and locality for *C. jorgei* and highlight inconsistencies in some *Contracaecum* spp. deposits based on sequence comparisons. Through morphometric analyses and comparative analyses of genetic sequences in GenBank, using a *locus* from the cytochrome oxidase subunit 2 gene, present specimens were identified as *C. jorgei*. Misidentified sequences of *Contracaecum* spp. were also observed and discussed. Thus, *C. jorgei* is reported for the first time in the Brazilian common snook *Centropomus undecimalis*, expanding the reports and, consequently, its geographic distribution in the Neotropical region.

Key words: common snook, *Contracaecum multipapillatum*, morphology, Rodrigo de Freitas lagoon, sequencing.

INTRODUCTION

The common snook *Centropomus undecimalis* (Bloch, 1792) is a euryhaline species distributed across the western Atlantic (Corrêa et al. 2010, Froese & Pauly 2024). This species is economically important throughout the Brazilian coast, which gives it good market acceptance and high commercial value (Rivas 1986).

Recently, a new anisakid species, *Contracaecum jorgei* Sardella, Mancini, Salinas, Simões & Luque, 2020, was described from the Argentine wolffish *Hoplias argentinensis* Rosso, Mabragaña, González-Castro, Bogan, Cardoso, Mabragaña, Delpiani & Díaz de Astarloa, 2018 from a shallow Pampean lake in central Argentina (Sardella et al. 2020).

In this context, this study reports common snooks *C. undecimalis* caught in the Rodrigo de Freitas lagoon in the State of Rio de Janeiro, as a new host and locality for *C. jorgei* which was identified by morphological and molecular methods, in addition to highlighting misidentifications of *Contracaecum* spp. deposited in GenBank that potentially hinder future taxonomic studies.

MATERIALS AND METHODS

Fifteen specimens of *C. undecimalis* (total length 56.5 cm [44.5-65.5 cm]; weight 1.510g [625–3.992g]) were collected by local fishermen in the Rodrigo de Freitas lagoon (22° 58' 16.02" S, 43° 12' 42.18" W), State of Rio de Janeiro, Brazil. These specimens were transported refrigerated to the Laboratório de Biologia e Ecologia de Parasitos (LABEPAR) of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) for parasitological analysis.

Host specimens were identified by Figueiredo-Filho et al. (2021) and necropsied to collection of parasites following the guidelines of Amato et al. (1991). The collected nematodes were stored in 70% ethanol. Later, some of them were transferred to temporary microscope slides in lactophenol and then analyzed morphologically according to Moravec (1998) and Sardella et al. (2020). Nematodes were observed, measured and photographed under an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Measurements are presented in millimeters with the average value shown in parentheses.

For genetic analysis, DNA was extracted from nematodes in 70% ethanol, using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. The PCR amplification of the cytochrome oxidase subunit 2 gene region (cox2) was carried out from two individuals as previously described by Marigo et al. (2015), with some modifications, using the primers 211F and 210R (Nadler & Hudspeth 2000). The amplicon was purified and sequenced as described by Duarte et al. (2020), using the PCR forward and reverse primers. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. and sequence compared with other nematodes available in the GenBank database, using the Basic Local Alignment Search Tool (BLAST). Multiple sequences were aligned using the ClustalW multiple alignments feature of MEGAX (v10.2.6), for the creation of a pairwise distance matrix based on the sequence of this study in comparison with sequences of related *Contracaecum* spp. (Kumar et al. 2018).

Field-collecting permits were issued by the Chico Mendes Institute for Biodiversity Conservation (Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio), through the Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade – SISBIO) under license number 77963, and Animal Ethics Committee (Comitê de Ética no Uso de Animais – CEUA/ICBS) of the UFRRJ under protocol no. 005/2019.

RESULTS

From fifteen specimens of *C. undecimalis* examined, ten were parasitized by third-stage anisakid larvae encysted in the abdominal cavity (Fig. 1a), totaling 31 specimens of *C. jorgei* collected. The prevalence was 66.7%, mean intensity 3.1±3.0 and mean abundance 2.1±2,9. The larvae were white in colour with cuticle striated transversely. Body length measured 11.17–18.33 (15.64) and width at the caecum level measured 0.27–0.38 (0.31). Cephalic extremity rounded with three underdeveloped lips, one lip with a small larval tooth (Fig. 1b). Excretory pore situated below the cephalic tooth 0.04–0.05 (0.045) in length. Nerve ring located from the anterior end, 0.19–0.29 (0.25) in length (Fig. 1b). Oesophagus measured 1.60–1.63 length × 0.06–0.16 wide (1.62 × 0.11). Ventriculus small and oval, 0.036–0.05 length × 0.049–0.05 wide (0.04 × 0.05). Intestine distinct and dark. Ventricular appendix measured 0.42 length × 0.06 wide. Intestinal caecum longer than ventricular appendix, 0.90–1.33 length × 0.10–0.15 wide (1.12 × 0.13). Posterior extremity with two anal glands and conical tail with pointed tip 0.11–0.14 (0.13) in length (Fig. 1c). This morphology was compatible with the recent description of L3 specimens of *C. jorgei* from *H. argentinensis* (Sardella et al. 2020). Voucher specimens were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), State of Rio de Janeiro, RJ, Brazil, in accordance with the number: 39665.

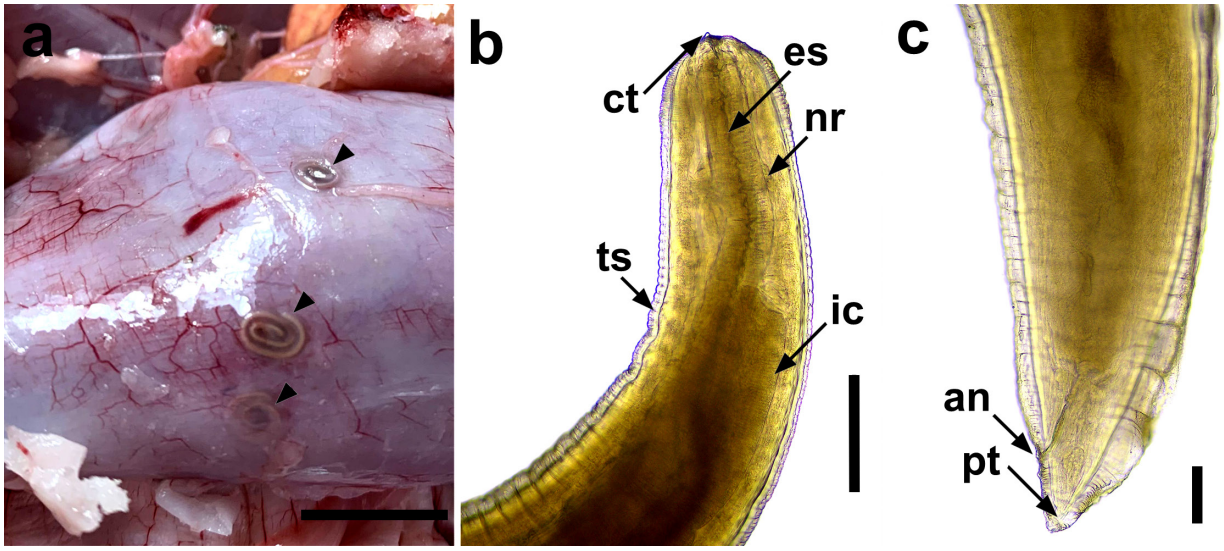

Figure 1. Specimens of the nematode *Contracaecum jorgei* of the abdominal cavity of common snook *Centropomus undecimalis*, from Rodrigo de Freitas lagoon, state of Rio de Janeiro, Brazil. 1a – Abdominal cavity of *Centropomus undecimalis* parasitized by *Contracaecum jorgei* encysted (arrowhead) in the peritoneum over the stomach. Scale bar: 10 mm. 1b – Anterior end of *Contracaecum jorgei* with cephalic tooth (ct), transverse striations (ts) on the cuticle, esophagus (es), nerve ring (nr) and intestinal cecum (ic). Scale bar: 0.20 mm. 1c – Posterior end of *Contracaecum jorgei* with anus (an) and conical tail with pointed tip (pt) (which is less visible due to the detachment of the cyst cover). Scale bar: 0.10 mm.

DNA amplification showed a clear band of 600 bp. The sequence obtained had 100% and 99.4% similarity with sequences of *C. jorgei* deposited in GenBank (MT304463 and MT304462, respectively); therefore, the molecular analysis corresponded with the morphological identification confirming that the third-stage larvae collected from *C. undecimalis* in this study are *C. jorgei*. A representative sequence of this study was deposited in the GenBank database under the accession number PP485039.

DISCUSSION

While the sequence from this study was 99-100% equivalent to *C. jorgei* sequences deposited in GenBank (Sardella et al. 2020), similarities ranging from 95-100% were observed for sequences (MH044672 to MH044685) identified as *Contracaecum multipapillatum* (Drasche, 1882) by Choc et al. (2020). In this study (Choc et al. 2020), third-stage larvae were collected from fishes of *Parachromis* spp., *Rhamdia* spp. and *Hoplias* spp. caught in rivers in the province of Guanacaste in Costa Rica, which were identified as *C. multipapillatum*. Sardella et al. (2020) carried out a detailed morphological study, both of larvae in intermediate hosts and adults in the definitive host, in addition to sequencing (MT304463 and MT304462) with genotypic differences of just 0.6% due to the substitution of 3 nucleotides (456/459). In contrast, Choc et al. (2020) reported third-stage larvae with few morphological details associated with many sequences (MH044672 to MH044685) that varied by more than 5% at the analysed *locus*. Therefore, it can be concluded that the deposits (MH044672 to MH044680; MH044682 to MH044685) in GenBank by Choc et al. (2020) which varied between 98.7-100% with the deposits of Sardella et al. (2020) must be *C. jorgei*, and the deposit MH044681 that had 5% dissimilarity must be another *Contracaecum* spp. The deposits of Sardella et al. (2020) date from September 2020

and therefore postdate the deposits of Choc et al. (2020), which are from May 2018; however, none of these inconsistencies or misidentifications in GenBank were discussed in Sardella et al. (2020).

The sequence correctly associated with the species *C. multipapillatum* for this *locus* of the *cox2* gene is probably that deposited (AF179910) by Nadler & Hudspeth (2000). In this study (Nadler & Hudspeth 2000), a phylogenetic study was carried out with several species of ascaridoid nematodes based on three genes, including *cox2*. In addition to this deposit, there are more sequences identified as *'affinis'* (*aff.*) for *C. multipapillatum* deposited in GenBank (EU852343 to EU852348). These were deposited by Mattiucci et al. (2009) in the description of two new *Contracaecum* spp., *Contracaecum gibsoni* Mattiucci, Paoletti, Solorzano & Nascetti, 2010 and *Contracaecum overstreeti* Mattiucci, Paoletti, Solorzano & Nascetti, 2010, originating from the so called *C. multipapillatum* complex. Anyway, these sequences identified as *Contracaecum aff. multipapillatum* by Mattiucci et al. (2009) are closer to the sequence (MG495095) of *C. overstreeti* (similarity of ~99%), than to the sequence (AF179910) deposited by Nadler & Hudspeth (2000) (similarity of ~93%).

By complementation, the pairwise distance matrix created based on the isolated sequence of this study (PP485039) with sequences deposited as *C. jorgei* and *C. multipapillatum* (Appendix) show that the sequence of this study has the closest relation with deposits related to *C. jorgei* (MT304463) and *C. multipapillatum* (MH044675), which were also closely related to each other. Furthermore, the remaining deposits identified as *C. multipapillatum* (MH044672 to MH044674; MH044676 to MH044680; MH044682 to MH044685) by Choc et al. (2020) were subsequently also related to *C. jorgei*. Thus, the pairwise distance matrix corroborates the afore mentioned observations about the inconsistencies in the identifications of these species deposited in GenBank, and that the deposits (MH044672 to MH044680; MH044682 to MH044685) from Choc et al. (2020) should be *C. jorgei*.

Species delimitation is not a generally consensual process and must undergo adjustments and reorganizations as studies advance, especially in an integrative taxonomy; therefore, it is worth highlighting the inconsistency in identifying distinct species that are genotypically closer than the same species, as observed in this study (Kunz 2002, Valkiūnas et al. 2008). As already evidenced by renowned authors (Valkiūnas et al. 2008), there is a low number of named species in GenBank and, worryingly, an increasing number of incorrectly identified species. Therefore, corrections of generic identifications or misidentifications become urgent, since there are few people in the next generation of scientists who are learning taxonomic skills to correctly associate morphological and molecular identifications (Valkiūnas et al. 2008). Furthermore, phylogenetic analyses, which arise from morphological and molecular studies, if based on misidentifications are likely to be misleading and result in erroneous conclusions (Valkiūnas et al. 2008).

In this sense, the deposits of Choc et al. (2020) reevaluated in this study widely expand the distribution and dispersion area of *C. jorgei* beyond Argentina (Sardella et al. 2020) to Costa Rica in Central America (Choc et al. 2020), also considering the Atlantic coast from South America, where *C. undecimalis* is widely distributed up to southeastern Brazil (current study). In fact, phylogenetically distant fish from which *C. jorgei* was collected in Sardella et al. (2020), Choc et al. (2020) and in this study, highlight its low specificity to the intermediate host, indicating a wide global distribution.

As a last point, the new host and locality records for *C. jorgei* warn of the potential risk of

human anisakiasis. The symptoms of anisakiasis are often unnoticed or confused with other gastrointestinal diseases, indicating that this disease is underestimated globally (Santos et al. 2020). In this context, this infection needs further studies and more comprehensive diagnoses, especially due to its increasing prevalence worldwide driven by new culinary trends (Santos et al. 2020).

In conclusion, in this study the anisakid *C. jorgei* was identified based on morphology and sequencing and recorded for the first time in the common snook *C. undecimalis* from southeastern Brazil. In addition, misidentifications were evidenced in GenBank, which directly hinder the taxonomic, phylogenetic and ecological studies of *Contracaecum* spp. and expands the list of known hosts and its known geographic distribution. Finally, this finding highlights the importance of continued research into parasitic infections in commercial fish populations, especially considering the potential impacts on public health and the fishing industry.

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APPENDIX

Appendix. Estimates of evolutionary divergence between sequences of *Contracaecum jorgei* from common snook *Centropomus undecimalis* and *Contracaecum* spp. sequences deposited in the GenBank database.

#mega
!Title: Phylogenetic Analysis;
!Format DataType=Distance DataFormat=LowerLeft NTaxa=24;
!Description
Analysis ==========
Analysis = ==========
Scope = Pairs of taxa
Estimate Variance = ==========
Variance Estimation Method = None
Substitution Model = ==========
Substitutions Type = Nucleotide
Model/Method = Maximum Composite Likelihood
Substitutions to Include = d: Transitions + Transversions
Rates and Patterns = ==========
Rates among Sites = Uniform Rates
Pattern among Lineages = Same (Homogeneous)
Data Subset to Use = ==========
Gaps/Missing Data Treatment = Pairwise deletion
Select Codon Positions = 1st,2nd,3rd,Non-Coding
No. of Sites = 619
d = Estimate
;
[1] #Contracaecum_jorgei(Centropomus_undecimalis)cox2
[2] #AF179910.1_Contracaecum_multipapillatum_cytochrome_oxidase_subunit_2_(cox-2)_gene_partial_cds_mitochondrial_gene_for_mitochondrial_product
[3] #EU852343.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[4] #EU852344.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial

[5] #EU852345.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB3_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[6] #EU852346.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB4_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[7] #EU852347.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB5_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[8] #EU852348.1_Contracaecum_aff._multipapillatum_B_SM-2008_isolate_CMB6_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[9] #MH044672.1_Contracaecum_multipapillatum_isolate_Ab_CRC1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[10] #MH044673.1_Contracaecum_multipapillatum_isolate_Ab_CRC2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[11] #MH044674.1_Contracaecum_multipapillatum_isolate_Can_CRC1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[12] #MH044675.1_Contracaecum_multipapillatum_isolate_Can_CRC2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[13] #MH044676.1_Contracaecum_multipapillatum_isolate_Hig_CRC1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[14] #MH044677.1_Contracaecum_multipapillatum_isolate_Hig_CRC2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[15] #MH044678.1_Contracaecum_multipapillatum_isolate_CP_GTM_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[16] #MH044679.1_Contracaecum_multipapillatum_isolate_Est_GTM1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[17] #MH044680.1_Contracaecum_multipapillatum_isolate_Est_GTM2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[18] #MH044681.1_Contracaecum_multipapillatum_isolate_EHig_GTM1_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[19] #MH044682.1_Contracaecum_multipapillatum_isolate_EHig_GTM2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[20] #MH044683.1_Contracaecum_multipapillatum_isolate_Mar_GTM_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
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[22] #MH044685.1_Contracaecum_multipapillatum_isolate_RD_GTM2_cytochrome_oxidase_subunit_II_(COII)_gene_partial_cds_mitochondrial
[23] #MT304462.1_Contracaecum_jorgei_cytochrome_oxidase_subunit_2_gene_partial_cds_mitochondrial
[24] #MT304463.1_Contracaecum_jorgei_cytochrome_oxidase_subunit_2_gene_partial_cds_mitochondrial
[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24]
[1]
[2] 0.085
[3] 0.075 0.053

[4] 0.071 0.058 0.008
[5] 0.075 0.056 0.005 0.008
[6] 0.076 0.055 0.004 0.009 0.001
[7] 0.073 0.052 0.004 0.009 0.004 0.003
[8] 0.075 0.053 0.003 0.008 0.003 0.001 0.001
[9] 0.004 0.085 0.074 0.071 0.074 0.076 0.073 0.074
[10] 0.003 0.083 0.073 0.069 0.072 0.074 0.071 0.073 0.009
[11] 0.006 0.093 0.081 0.078 0.081 0.083 0.080 0.081 0.006 0.009
[12] 0.000 0.085 0.074 0.071 0.074 0.076 0.073 0.074 0.006 0.003 0.006
[13] 0.003 0.089 0.076 0.073 0.076 0.078 0.074 0.076 0.006 0.006 0.006 0.003
[14] 0.001 0.083 0.073 0.069 0.072 0.074 0.071 0.073 0.007 0.004 0.007 0.001 0.004
[15] 0.004 0.085 0.078 0.074 0.078 0.080 0.076 0.078 0.010 0.004 0.010 0.004 0.007 0.006
[16] 0.007 0.089 0.078 0.074 0.078 0.080 0.076 0.078 0.009 0.012 0.012 0.009 0.009 0.010 0.013
[17] 0.007 0.089 0.078 0.074 0.078 0.080 0.076 0.078 0.009 0.012 0.012 0.009 0.009 0.010 0.013 0.000
[18] 0.036 0.078 0.055 0.052 0.055 0.057 0.053 0.055 0.036 0.033 0.043 0.036 0.040 0.038 0.038 0.043 0.043
[19] 0.007 0.078 0.078 0.078 0.081 0.080 0.076 0.078 0.013 0.007 0.013 0.007 0.010 0.009 0.006 0.013 0.013 0.041
[20] 0.004 0.081 0.074 0.071 0.074 0.076 0.072 0.074 0.010 0.004 0.010 0.004 0.007 0.006 0.006 0.013 0.013 0.038 0.009
[21] 0.004 0.083 0.076 0.073 0.076 0.078 0.074 0.076 0.007 0.004 0.007 0.004 0.004 0.006 0.009 0.007 0.007 0.038 0.009 0.009
[22] 0.006 0.080 0.072 0.069 0.072 0.074 0.071 0.072 0.012 0.003 0.012 0.006 0.009 0.007 0.007 0.015 0.015 0.037 0.010 0.004 0.007
[23] 0.005 0.083 0.075 0.071 0.075 0.077 0.073 0.075 0.008 0.008 0.011 0.005 0.008 0.006 0.009 0.012 0.012 0.035 0.012 0.009 0.009 0.011
[24] 0.000 0.083 0.074 0.071 0.074 0.076 0.073 0.074 0.006 0.003 0.006 0.000 0.003 0.001 0.004 0.009 0.009 0.037 0.007 0.004 0.004 0.006 0.005

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