

Morphology and molecular analysis of *Goezia spinulosa* (Nematoda: Raphidascarididae) from arapaimas in the northwestern Brazil

Maralina Torres da Silva^{1,5}, Pedro Hercílio de Oliveira Cavalcante^{2,5}, Ana Carolina Alves de Camargo³, Vanessa Aparecida das Chagas Moutinho³, Everton Gustavo Nunes dos Santos^{3,4}, Cláudia Portes Santos³

1Instituto Federal do Acre – IFAC, Rio Branco, AC, Brazil.

2Instituto Federal do Acre – IFAC, Xapuri, AC, Brazil.

3Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brazil.

4Fundação Instituto de Pesca do Estado do Rio de Janeiro – FIPERJ, Escritório Regional Metropolitano II, Duque de Caxias, RJ, Brazil.

5Programa de Pós-Graduação em Biodiversidade e Saúde, Instituto Oswaldo Cruz, Fiocruz.

Arapaima gigas, a fish with a high market value, has been farmed in different localities within Brazil. Among its parasites, adults of *Goezia spinulosa* are reported to cause ulcers in the stomach and to result in the death of farmed fingerlings. Due to the veterinary importance of *G. spinulosa* in farmed arapaimas, an integrative taxonomic study, including morphological, ultrastructural and genetic data, was undertaken in order to better characterize this nematode and thus contribute to a better specific diagnosis of adult and larval stages. The 64 fish obtained from semi-intensive fish farming in Acre State, Brazil were examined in saline medium under a stereomicroscope. Parasites were fixed in 70% ethanol or 4% formaldehyde solution. Some specimens were cleared and examined as temporary mounts using glycerine. Drawings were made with the aid of a drawing tube. Some specimens were post-fixed in 1% OsO₄ and potassium ferrocyanide 0.8%, dehydrated in alcoholic series, and conducted to critical point with CO₂ coated with gold and visualized through scanning electron microscopy. Genomic DNA of the parasites was extracted using QIAmp DNA Mini Kit and amplified by PCR using different set of primers to be sequenced. The fish measured 7–42 cm in total length and weighed 2–392 g. The prevalence of *G. spinulosa* was 29.69% and the intensity of infection was 1–60 parasites per fish. The site of infection was mainly the stomach, less often the intestine. Morphological and ultrastructural analyses of *G. spinulosa* showed the importance of its spiny body in firmly attaching the worm to the gastric mucosa, resulting in lesions, ulcers and deep gastric perforations of the stomach wall. New sequences for partial 18S rDNA, ITS1, 5.8S and ITS2 rDNA, partial 28S rDNA, cox1 mtDNA and for cox2 mtDNA are presented. Phylogenetic reconstructions based on the partial 18S and 28S rDNA shows species of *Goezia* occur in a clade well separated from other genera in both analyses. Both the partial 18S and 28S rDNA genes represented good genetic markers for distinguishing genera of the Raphidascarididae, with exception of *Hysterothylacium*. This integrated taxonomic study produced a robust profile for *G. spinulosa* that will aid the diagnosis of both adults and larval stages from arapaimas and their possible intermediate hosts. This study was supported financially by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior, Fundação Oswaldo Cruz, Instituto Federal do Acre and Fundação de Amparo à Pesquisa do Acre.

Keywords: *Arapaima gigas* – Nematoda - *Goezia spinulosa*