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ELAINE LOPES DE CARVALHO

SISTEMÁTICA E INTERAÇÃO PARASITO-HOSPEDEIRO DE HELMINTOS DE *Phalacrocorax brasilianus* E *Cairina moschata domestica* DA RESERVA EXTRATIVISTA MARINHA DE SOURE, ILHA DE MARAJÓ, PARÁ

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Defesa de tese apresentada à Universidade Federal Rural da Amazônia, como parte das exigências do Curso de Doutorado em Saúde e Produção Animal na Amazônia: área de concentração Saúde e Meio Ambiente.

Orientadora: Profa. Dra. Elane Guerreiro Giese

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SUMÁRIO

RESUMO

A ordem Anseriforme tem como um dos representantes *Cairina moschata domestica*, conhecido como pato doméstico de grande importância econômica, possuem hábito aquático, e a maioria das espécies são migratórias para suprir as necessidades de alimento, nidação, abrigo e muda. O Brasil possui poucas espécies de anatídeos. A ordem Suliformes inclui quatro famílias, cujos representantes são encontrados em todos 39 os continentes, sendo um deles o *Phalacrocorax brasilianus*. Ambos vivem em ambiente em comum na Ilha de Marajó, onde há uma variável quantidade de hospedeiros intermediários que fazem parte de ciclos biológicos de parasitas dessas aves. Amostras de *C. moschata* e *P. brasilianus* provenientes da Ilha de Marajó, Estado do Pará, foram analisadas. Essas amostras serão obtidas das aves mortas de domínio da população local. Os helmintos parasitos encontrados serão processados para análise por microscopia de luz, microscopia eletrônica de varredura, histologia e biologia molecular. Serão utilizados catálogos, chaves de identificação, livros e artigos científicos com descrições originais e redescrição de espécies para identificação do táxon. Os mesmos foram quantificados sob estéreomicroscópio, para determinação dos parâmetros de prevalência, intensidade média de infecção e abundância média. Os dados foram tabulados e comparados com os presentes na literatura existente para cada táxon identificado. Com isso, identificamos espécies de helmintos, caracterizamos a relação parasita-hospedeiro prejudiciais a essas aves, adicionando assim dados sobre a biodiversidade parasitária de aves no norte do Brasil.

Palavras-chave: *Cairina*. *Phalacrocorax*. Helmintos. Parasitos.

ABSTRACT

The Anseriforme order has as one of the representative *Cairina moschata domestica*, known as Muscovy duck of great economic importance, they have an aquatic habit, and most species are migratory to meet the needs of food, nesting, shelter, and molting. Brazil has few species of anatidae. The order Suliformes includes four families, whose representatives are found on all 39 continents, one of them being the *Phalacrocorax brasilianus*. Both live in a common environment on the Marajó Island, where there is a variable number of intermediate hosts that are part of the biological cycles of these birds' parasites. Samples of *C. moschata* and *P. brasilianus* from Marajó Island, State of Pará, were analyzed. These samples will be obtained from dead birds in the domain of the local population. The parasitic helminths found will be processed for analysis by light microscopy, scanning electron microscopy, histology, and molecular biology. Catalogs, identification keys, books and scientific articles with original descriptions and species redescription will be used to identify the taxon. They will be counted, under a stereomicroscope, to determine the parameters of prevalence, mean infection intensity and mean abundance. Data will be tabulated and compared with data in the existing literature for each identified taxon. With this, we identified species of helminths, characterized the parasite-host relationship harmful to these birds, thus adding data on the parasite biodiversity of birds in northern Brazil.
Keywords: *Cairina*. *Phalacrocorax*. Helmintes. Parasites.

1. CONTEXTUALIZAÇÃO

1.1. Biodiversidade na Amazônia brasileira

A Amazônia possui uma biodiversidade sustentada por diferentes níveis taxonômicos (HASEYAMA; CARVALHO, 2011). Um dos ambientes que contribui para a alta diversidade na região amazônica são os ecossistemas aquáticos que abrigam um conjunto de interações ecológicas onde a variação periódica do nível das águas é um fator determinante para comunidade de organismos aquáticos presente em rios com planícies alagadas (WELCOMME, 1985; JUNK *et al*., 1989). Essas alterações do nível das águas promovem modificações tanto bióticas e abióticas (LOWE-MCCONNELL, 1999).

Os períodos sazonais na Amazônia são diferenciados pelas características limnológicas durante dois períodos extremos, a das águas baixas e das águas altas (JUNK *et al*., 1989). A influência desse fenômeno pode causar grandes alterações nos processos ecológicos dos lagos de inundação ao longo deste ciclo sazonal (BITTENCOURT; AMADIO, 2007). O aparecimento de áreas alagáveis aparece como uma das maiores forças controladoras da dinâmica dos ecossistemas aquáticos (SIOLI, 1985). Conforme SANTOS (2006) as áreas úmidas são influenciadas pelo regime das marés, dos rios e das chuvas, e servem como criadouros para várias espécies de animais (aves, mamíferos, insetos etc.) e área de desova para organismos aquáticos (peixes, crustáceos etc.). Dessa forma, essa dinâmica influencia significativamente a vida dos peixes e outros organismos aquáticos, incluindo a fauna de parasitos dos peixes (PANTOJA, 2013).

No nível individual, as relações entre um vetor e o microrganismo que ele veicula ou transporta podem variar desde a simples relação de indivíduos de espécies diferentes à intimidade essencial, quando uma fase do desenvolvimento do microrganismo se desenvolve no corpo do hospedeiro intermediário (ÁVILA-PIRES, 1989).

> As relações de parasitismo, comensalismo e simbiose sugerem etapas de uma longa sequência evolutiva, de acomodação e adaptação mútuas, durante a história filogenética dos organismos envolvidos. Entretanto, nem sempre isso se verifica, como o demonstra a variação na reação do hospedeiro à colonização. Ao nível do ecossistema, a situação desse equilíbrio traduz-se nas epizootias ou epidemias e nas enzootias ou endemias. A condição enzoótica ou endêmica representa o estágio de adaptação alcançado através do processo de seleção natural, que elimina os hospedeiros mais suscetíveis e os micro-organismos mais patogênicos, em cada comunidade biótica (ÁVILA-PIRES, 1989).

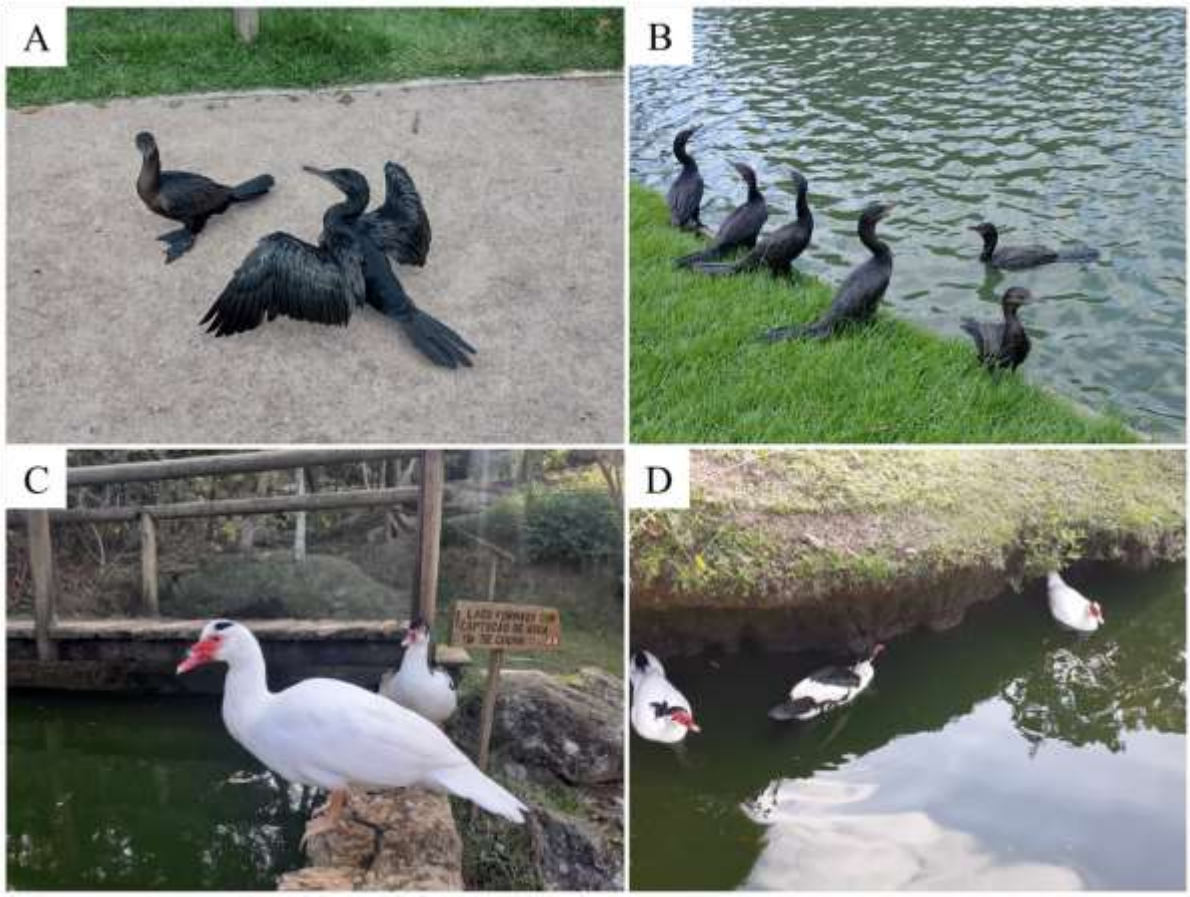
As aves desempenham importantes funções biológicas nestes ambientes aquático litorâneo e continental onde são consideradas predadores de topo da cadeia alimentar (CONDE-TINCO; IANNACONE, 2013). Seu comportamento de forrageio ocorre com a disponibilidade de presas e as condições ambientais (PETRY *et al*., 2008, 2009, 2010). Dessa forma, consideram-se essas aves como bioindicadoras nestes ecossistemas aquáticos (SICK, 1997; BARRETT *et al*., 2007; BARQUETE *et al*., 2008).

O estudo da helmintofauna parasitária de aves aquáticas segundo KENNEDY *et al*., 1986 é considerado de grande importância, devido à função da dispersão de uma grande variedade de espécies de helmintos que atuam no ecossistema, ajudando-os a conquistar novos habitats, fazendo com que essas aves possuam comunidades de parasitas diversificado, por estar em proximidade às águas, onde se alimentam.

Os estudos de biodiversidade parasitária baseiam-se na importância desses organismos como causadores de doença influenciando negativamente na saúde dos habitats silvestre e domiciliar, trazendo um novo conceito ao entendimento das interações ecológicas, dos padrões de distribuição dos hospedeiros e da história de muitas regiões (BROOKS; HOBERG, 2000; BAUTISTA-HERNÁNDEZ *et al*., 2015).

As aves da família Anatidae e Phalacrocoracidae (Figura 1) embora sejam de diferentes famílias, habitam a Ilha de Marajó em regiões com áreas alagadas, várzeas, igarapés e praias, em busca de alimentos e reprodução, podendo dessa forma ocorrer a propagação de helmintos parasitos que fazem parte do ciclo biológico de cada espécie parasita dessas aves. A compreensão dos aspectos taxonômicos e ecológicos desses parasitos tem grande importância prática por razões veterinárias, de conservação em geral e saúde pública.

Figura 1 - Representantes da família Phalacrocoracidae e Anatidae. A-B. *Phalacrocorax brasilianus*. C-D. *Cairina moschata domestica*.



Fonte: Carvalho (2023).

1.2. *Cairina moschata domestica*

A ordem Anseriformes é formada por 170 espécies de aves aquáticas distribuídas nas famílias Anhimidae, Anseranatidae e Anatidae, onde está última tem como um dos representantes a espécie *Cairina moschata* (ASHTON; ASHTON, 2001; SIGRIST, 2009; SILVEIRA, 2012). São animais amplamente usados como fonte de proteínas por diversos povos (GOIS *et al*., 2012; FEHLBERG, 2015). Os patos domésticos (*Cairina moschata domestica*) são criados extensivamente na maior parte do Brasil, e na Ilha do Marajó são de grande importância à população local como fonte de alimento, conhecer sua fauna parasitária é importante para política de saúde pública e evitar possíveis zoonoses, pois existe registro de que os patos domésticos são hospedeiros acidentais para grande parte de espécies de larvas, e hospedeiros definitivos para algumas das espécies de helmintos parasitos encontrados. (CARVALHO *et al*., 2019; 2020).

Santana (2019) demonstrou que essas aves possuem uma ampla comunidade parasitária, podendo estar relacionado ao sistema de criação adotado pelos proprietários destes animais,

devido a coabitação com outras espécies domésticas e ao contato livre com animais silvestres, predispondo ao surgimento de infecções parasitárias.

Os patos são aves de grande rusticidade e com reduzida propensão a doenças, desde que mantidos num ambiente higiênico e isento de umidade, porém, particularmente propensos a infecção por helmintos (GOWER,1939). São aves aquáticas que possuem os dígitos dos pés interligados por membranas de forma a funcionarem como um par de remos. Suas penas espessas as protegem do frio e facilita a flutuação, a maioria das espécies são migratórias para suprir as necessidades de alimento, nidação, abrigo e muda (SICK, 2001; QUINALHA *et al*., 2011).

A alimentação, quando criados extensivamente, varia por meio da ingestão de plantas, raízes, pequenos peixes, caracóis e insetos, sendo necessário a suplementação caso se encontrem em fase de postura, ou sejam criados para corte, podendo-se fornecer arroz, milho, derivados da mandioca, batata doce e outras fontes de alimentos (MEULEN; DIKKEN, 2003).

1.3. *Phalacrocorax brasilianus*

A ordem Suliformes inclui quatro famílias, Fregatidae Degland & Gerbe, 1867, Sulidae Reichenbach, 1849, Anhingidae Reichenbach, 1849 e Phalacrocoracidae Reichenbach, 1849 cujos representantes são encontrados em todos os continentes (MONTEIRO, 2006). A espécie *Phalacrocorax brasilianus* (Gmelin, 1789) (Sin. *Nannopterum brasilianus*), popularmente conhecido como "biguá", possui distribuição no sul dos EUA e toda América do Sul (BIRDLIFE INTERNACIONAL, 2016); habita águas interiores e em todo o litoral, rios, lagos, estuários e manguezais, mas não se afastam da costa (SICK, 1997; SOUZA *et al*., 2008).

É uma das poucas aves que habitam ambientes de água doce e salina (QUINTANA; YORIO; BORBOROGLU, 2002). Esta espécie é difundida no Brasil e habita as margens de lagoas, rios e baías. A ave possui 63–68 cm de comprimento e peso em torno de 1,3kg, possui pescoço longo, cabeça pequena, bico cinzento longo e fino, sendo a ponta da maxila terminal em forma de gancho preto com coloração marrom volta, pele facial amarela com bordas finas e brancas e algumas penas brancas na cabeça e pescoço durante a reprodução (ROSÁRIO, 1996; SICK, 1997; GWYNNE *et al*., 2010).

Esta espécie piscívora é bastante ativa durante o dia e pode ser encontrado isolado ou coletivamente, facilitando seu monitoramento (SICK, 1997). Seus ninhos são construídos sobre árvores, em áreas alagadas ou nas proximidades de ambientes aquáticos (ROSÁRIO, 1996; SICK, 1997). Os peixes são o principal componente da sua dieta que é complementada por

anfíbios, crustáceos, moluscos, répteis e até pequenos mamíferos, mas o conhecimento sobre a identificação das espécies que constituem a sua dieta ainda é escasso (WELLER, 1999; AZPIROZ, 2001; VIOLANTE-GONZÁLEZ *et al*., 2011; DE OLIVEIRA *et al*., 2019).

O comportamento dessas aves pode ser modulado por variações sazonais, com a mudança de períodos e volume de chuva. Essas espécies piscívoras, podem ser afetadas pela disponibilidade de peixes durante as estações seca e chuvosa. Em períodos de seca, quando há pouca precipitação e alta evaporação, o nível da água dos ambientes aquáticos diminui, expondo uma grande quantidade de presas mortas a essas aves. Em épocas com alta precipitação, o nível da água aumenta, dificultando a busca por esses (GUIBU *et al*., 2007).

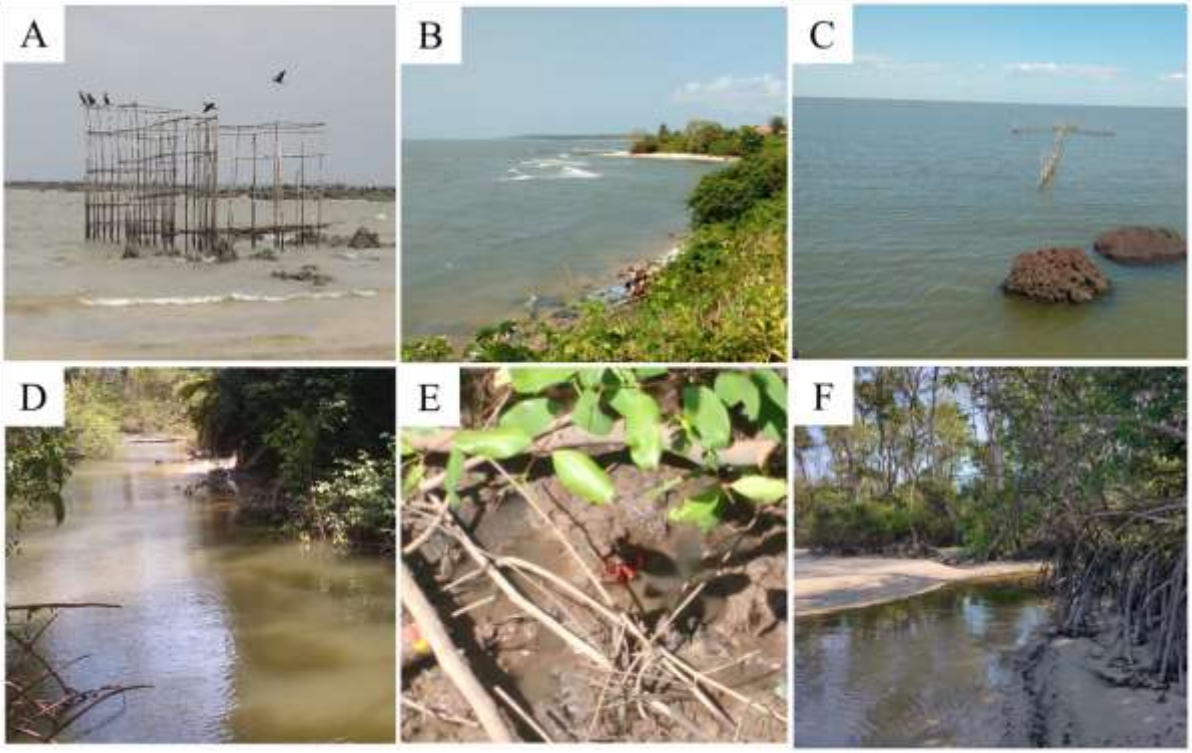
Em estudos sobre a diversidade parasitária de *P. brasilianus*, Monteiro *et al*. (2011), realizaram análise da estrutura das comunidades de parasitos nas aves, encontrando 20 espécies de parasitos, assim tem despertado muito interesse devido à ampla distribuição geográfica do gênero *Phalacrocorax* Brisson, 1760. Os estudos nas áreas onde são encontradas, revelaram uma helmintofauna extremamente rica, sendo reflexo de um ambiente complexo, rico em invertebrados e vertebrados que atuam como hospedeiro intermediário nos ciclos biológicos para as diferentes espécies de parasitos (MONTEIRO, 2006). *P. brasilianus* são importantes agentes de disseminação de parasitas devido ao seu hábito migratório (KENNEDY, 1998). Porém no Brasil, a helmintofauna ainda não é bem definida.

1.4. Ilha de Marajó, Pará

A ilha de Marajó, é a maior ilha do arquipélago na foz do rio Amazonas, com 50.000 km² de extensão, situada a nordeste do Estado do Pará, é banhada pelas águas brancas do rio Amazonas, Baia do Marajó; rio Tocantins e pelo Oceano Atlântico, sendo um dos estuários mais relevantes do Brasil (OTCA, 2012). O município de Soure com área de 2.857 km² de extensão, e população está estimada em 25.565 pessoas (IBGE, 2018).

As paisagens naturais do Marajó (Figura 2) são formadas por extensas áreas de terra firme, várzea, igapó, manguezais com influência marinha e campos naturais que podem ser sazonalmente inundáveis devido a vasta rede hidrográfica da região, caracterizada por emaranhados de canais, furos, baías, lagos, igarapés, praias de mar e rio, com a vegetação influenciada diretamente pela hidrografia, que define os principais ecossistemas regionais (JAPIASSÚ; FILHO, 1974; BRASIL, 2007).

Figura 2 - Paisagens da Ilha de Marajó. A, B, C são regiões de praia com influência das águas de rio e de mar com presença de currais de pesca. D, E e F área de igarapé, manguezal.



Fonte: Carvalho (2023).

1.5. Espécies parasitos de *Cairina moschata domestica*

Mattos Junior *et al*. (2008), identificaram uma biota parasitária composta por uma variedade de nematódeos em patos do Rio de Janeiro, e atualmente pesquisas mais recentes registraram a ocorrência dos parasitos *Eucoleus contortus* e *Anisakis* sp. no esôfago dessas aves, conforme Carvalho *et al*., (2019; 2020). E Bruno *et al*. (2021) por meio de exames coproparasitológico de anatídeos oriundo do estado de São Paulo, identificaram as Ordens Trichuroidea e Ascaridia. Nas tabelas 1 e 2 há a apresentação atualizada dos helmintos parasitos de *C. moschata domestica* no exterior e no Brasil.

Tabela 1- Helmintos parasitos de *C. moschata* de acordo com os registros feitos por diferentes pesquisadores no exterior.

Helmintos	Sitio de infecção	Localidade	Referências
Filo Nematoda **Superfamília Habronematoidea Ivaschkin, 1961** **Família Tetrameridae Travassos, 1914**			
Tetrameres fissipina (Diesing, 1860) Travassos, 1914	Proventrículo	Índia	Kamil *et al*., 2011
Superfamília Heterakoidea Railliet e Henry, 1914 **Família Heterakidae Railliet e Henry, 1914**			
Heterakis gallinarum (Schrank, 1788)	Cecos	África, Tanzânia	Alexander e Mclaughlin, 1997; Muhairwa *et al*., 2007
H. díspar (Schrank, 1790)		Tanzânia	Muhairwa *et al*., 2007
H. isolonche Linstow, 1906		Tanzânia	Muhairwa *et al*., 2007
Família Ascariidae Travassos, 1919			
Ascaridia galli (Schrank, 1788) Freeborn, 1923	Intestino	África	Alexander e Mclaughlin, 1997; Muhairwa *et al*., 2007
A. columbae (Gmelin, 1979)		Tanzânia	Muhairwa *et al*., 2007
A. dissimilis Vigueras, 1931		Tanzânia	Muhairwa *et al*., 2007
Superfamília Spiruroidea Oerley, 1885 **Família Gongylonematidae Sobolev, 1949**			
Gongylonema congolense Fain, 1955	Papo, esôfago	África	Alexander e Mclaughlin., 1997
Superfamília Subuluroidea Travassos, 1930 **Família Subuluroidae Yorke e Maplestone, 1926**			
Subulura brumpti (Lopez-Neyra, 1922)	Cecos		Muhairwa *et al*., 2007
Subulura strongylina (Rudolphi, 1819)			

Subulura suctoria (Molin, 1860)	Intestino	Tanzânia	Muhairwa *et al*., 2007
Superfamília Thelazioidea **Família Thelaziidae (Skrjabin, 1915) Railliet, 1916**			
Oxyspirura parovatum Sweet, 1910	Olhos	Austrália	Gower, 1939
Superfamília Acuarioidea Molin, 1860 **Família Acuariidae Seurat, 1913**			
Streptocara incognita Gibson, 1968	Esôfago	Itália	Bano *et al*., 2005
Superfamília Trichinelloidea Railliet, 1916 **Família Capillariidae Neveu-Lemaire, 1936**			
Eucoleus contortus (Creplin, 1839) *Eucoleus annulatus* (Molin, 1858) *Capillaria anatis* (Schrank, 1790)	Cecos	Tanzânia	Muhairwa *et al*., 2007
Superfamília Habronematoidea Railliet e Henry, 1915 **Família Habronematidae Chitwood e Wehr, 1932**			
Parhadjelia cairinae Zhang e Brooks, 2005	Papo	Costa Rica	Zhang e Brooks, 2005
Filo Platyhelminthes, Classe Trematoda **Família Cyclocoelidae Stossich, 1902**			
Typhlocoelum cucumerinum (Rudolphi, 1809)	Fossas nasais, Traqueia	Colômbia	Hoyos *et al*., 2017, Assis *et al*., 2021
Superfamília Opisthorchioidea Looss, 1899 **Família Opisthorchiidae Looss, 1899**			

Amphimerus anatis (Yamaguti, 1933)	–	Japão, China	Gower, 1939
Filo Platyhelminthes, Classe Cestoda **Superfamília Cyclophyllidea** **Família Hymenolepididae Ariola, 1899**			
Hymenolepis papillata Fuhrmann, 1906 *Sobolevicanthus bisaccata* (Fuhrmann, 1906)	Intestino	–	Gower, 1939
Família Paruterinidae Fuhrmann, 1907			
Biuterina longiceps (Rudolphi, 1819) Fuhrmann, 1908	–	–	Gower, 1939
Família Davaineidae Braun, 1900			
Raillietina echinobothrida (Megnin, 1881) *Raillietina tetragona* (Molin, 1858)	Intestino	Tanzânia	Muhairwa *et al*., 2007

Fonte: Carvalho (2023).

Tabela 2 - Helmintos parasitos de *C. moschata domestica* de acordo com os registros feitos por diferentes pesquisadores no Brasil.

Helmintos	Sitio de infecção	Localidade	Referências
Filo Nematoda			
Superfamília Habronematoidea Ivaschkin, 1961			
Família Tetrameridae Travassos, 1914			
Tetrameres fissipina (Diesing, 1860) Travassos, 1914	Proventrículo	Minas Gerais, Rio de janeiro	Vicente *et al*., 1995; Mattos Junior *et al*., 2008
Tetrameres sp. Creplin, 1846	Proventrículo	Pará, Goiás	Vicente *et al*., 1995; Machado *et al*., 2006
Superfamília Heterakoidea Railliet e Henry, 1914			
Família Heterakidae Railliet e Henry, 1914			
Heterakis sp. Dujardin, 1844	Cecos	Goiás	Machado *et al*., 2006
H. gallinarum (Schrank, 1788)	Cecos	Distrito Federal, Minas Gerais, Goiás	Vicente *et al*., 1995; Machado *et al*., 2006
Superfamília Subuluroidea Travassos, 1930			
Família Subuluroidae Yorke e Maplestone, 1926			
Subulura sp. Molin, 1860	Intestino, cecos	Mato Grosso do Sul, Goiás	Vicente *et al*., 1995; Machado *et al*., 2006
Família Anisakidae Railliet & Henry, 1912			
Anisakis sp. Dujardin, 1845	Esôfago	Pará	Carvalho *et al*., 2021
Superfamília Trichinelloidea Railliet, 1916			
Família Capillariidae Neveu-Lemaire, 1936			
Eucoleus cairinae (Freitas e Almeida, 1935)	Esôfago	Paraná, Rio de Janeiro	Vicente *et al*., 1995; Mattos Junior *et al*., 2008
Eucoleus contortus Creplin, 1839 (Gagarin, 1951)	Esôfago	Pará	Carvalho *et al*., 2019
Capillaria phasianina (Kotlán,1914)	Esôfago e cecos	Rio de Janeiro	Mattos Junior *et al*., 2008

Capillaria sp. (Pinto e Almeida, 1935)	Esôfago, cecos, vesícula biliar	Brasil, Rio de Janeiro	Vicente *et al*., 1995; Mattos Junior *et al*., 2008
Capillaria cairina Carvalho, Santana, Sindeaux Neto, Silva e Giese 2023	Esôfago	Pará	Carvalho et al., 2023a
Superfamília Habronematoidea Railliet e Henry, 1915 Família Habronematidae Chitwood e Wehr, 1932			
Hadjelia neglecta (Lent e Freitas, 1939; Chabaud, 1975)	Proventrículo, moela e cecos	Goiás, Rio de Janeiro	Vicente *et al*., 1995; Machado *et al*., 2006; Mattos Junior *et al*., 2008
Família Syngamidae Leiper, 1912			
Syngamus trachea (Montagu, 1811)	traqueia	Marajó, Pará	Carvalho *et al*., 2021
Filo Platyhelminthes, Classe Trematoda Família Echinostomatidae Looss, 1899			
Echinostoma revolutum (Froelich, 1902)	Bolsa cloacal, intestino	Goiás, Rio de Janeiro	Lima, 1980; Machado *et al*., 2006; Mattos Junior *et al*., 2008
E. mendax Dietz, 1909	Intestino	Goiás	Machado *et al*., 2006
Família Cyclocoelidae Stossich, 1902			
Typhlocoelum cucumerinum (Rudolphi, 1809)	Fossas nasais e traqueia	Rio de Janeiro, Goiás	Travassos, 1921; Lima, 1980; Machado *et al*., 2006
Ophthalmophagus magalhaesi Travassos, 1921	Fossas nasais e traqueia	Rio de Janeiro, Goiás	Travassos, 1921; Lima, 1980; Machado *et al*., 2006
Família Eucotylidae Cohn, 1904			
Eucotyle freitasi Costa & Freitas, 1972	-	Rio de Janeiro	Lima, 1980
Família Prosthogonimidae (Lühe, 1899) Lahille, 1922			
Prosthogonimus sp. (Lühe, 1899) Markov, 1903	Bolsa de Fabricius e oviduto	Goiás	Machado *et al*., 2006
Prosthogonimus ovatus (Rudolphi, 1803) Lühe, 1899	-	Rio de Janeiro	Lima, 1980

Família Zygocotylidae Ward, 1917			
Zygocotyle lunata (Diesing, 1836) Stunkard, 1917	Cecos	Rio de Janeiro, Goiás	Lima, 1980; Machado *et al.*, 2006
Filo Platyhelminthes, Classe Cestoda Superfamília Cyclophyllidea Família Hymenolepididae Ariola, 1899			
Fimbriaria fasciolaris (Pallas,1781; Frolich, 1802)	Jejuno	Rio de Janeiro	Mattos Junior *et al*., 2008
Família Dilepididae Railliet e Henry, 1909			
Lateriporus sp. Fuhrmann, 1907	Jejuno	Rio de Janeiro	Mattos Junior *et al*., 2008

Fonte: Carvalho (2023).

1.6. Espécies parasitos de *Phalacrocorax brasilianus*

No Brasil a pesquisa de Monteiro *et al*. (2011) sobre helmintos parasitos de *P. brasilianus*, observaram que todas as aves examinadas estavam infectadas, e os número de espécies de helmintos por hospedeiro variou de 2 a 14. O número crescente de aves Phalacrocoracidae em associação com muitas espécies de organismos aquáticos (peixes, caracóis do gênero *Lymnaea* etc.) dentro do território da Rússia são uma possível razão para a epizootia causada por espécies de parasitos invasores, gerando a possibilidade de disseminação desses parasitos para outros hospedeiros (YAKOVLEVA *et al*. 2020). Além disso, também é possível que algumas dessas espécies de parasitos tenham sido introduzidas no país por meio da migração das aves parasitadas que chegam sazonalmente para passar o verão no Hemisfério Sul (MARTÍNEZ-SALAZAR *et al*., 2016).

No Chile Torres *et al*. (2005) observaram que os filhotes de *P. brasilianus* da natureza são afetados por pelo menos quatro espécies de nematódeos no trato digestivo e provavelmente adquiriram a infecção através dos alimentos regurgitados pelos pais, portanto adultos e filhotes devem abrigar essencialmente a mesma comunidade de nematódeos. No México Violante-González *et al*. (2011) analisaram a estrutura da comunidade parasitária dessa ave da região neotropical de duas lagoas (Coyuca e Tres Palos) do estado de Guerrero, México, registrando, quatorze espécies de helmintos adultos (6.391 identificados) de 48 biguás: 9 digeneos, 1 acantocéfalo, 1 cestoda e 3 nematódeos.

Na Índia *Echinostoma valentini* foi registrado por Sanjota e Ghazi, (2011) em *Phalacrocorax fuscicollis*, e na pesquisa de Bushra *et al*. (2019) elaboraram uma lista de verificação de parasitas helmintos de aves no Paquistão, onde registraram em Phalacrocoracidae trematódeos e nematódeos. Sendo a maioria desses helmintos parasitas do trato digestivo, mas alguns registrados em outros órgãos, como traqueia, olho ou cérebro.

Para melhor entendimento da helmintofauna Violante-González *et al*. (2015) sugerem realizar um maior número de estudos dessas mesmas espécies de aves ictifágicas, assim como das outras espécies migratórias ou residentes, que habitam temporária ou permanentemente a área a ser estudada, para tentar conhecer uma parte importante do ciclo de vida de muitas espécies de parasitas alogênicos. Na tabela 3 abaixo estão listadas as espécies de helmintos parasitos de Phalacrocoracidae já descritos.

Tabela 3 - Quadro de helmintos parasitos de aves da Família Phalacrocoracidae no Brasil e no exterior.

Helmintos	Sitio de infecção	Localidade	Referências
Filo Nematoda **Família Dioctophymatidae Castellani & Chalmers, 1910**			
Eustrongylides sp. (larva)	Esôfago	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Família Desmidocercidae Cram, 1927			
Desmidocercella incognita Ssolonitzin, 1932	Sacos aéreos e pulmões	Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Superfamília Ascaridoidea Baird, 1853 **Família Anisakidae Railliet & Henry, 1912**			
Anisakis Dujardin, 1845	Estômago	Chile	González-Acuña *et al*. 2020
Contracaecum rudolphii Hartwich, 1964	Proventrículo, Esôfago, estômago	Guaíba, RS; Berlim, Alemanha; Chile	Amato *et al*., 2006; Monteiro *et al*., 2011 Moravec & Scholz, 2016; González-Acuña D *et al*. 2020
Contracaecum jorgei n. sp**.**	Intestino e proventrículo	Argentina	Sardella *et al*. 2020
Contracaecum multipapillatum Drasche, 1882	Estômago, intestino	México	Violante-González *et al*., 2011
Família Syngamidae Leiper, 1912			
Syngamus sp.	Traqueia	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Syngamus trachea (Montagu, 1811) Chapin 1925	Esôfago	México	Violante-González *et al*., 2011
	Traqueia	Chile	Oyarzún-Ruiz & Muñoz-Alvarado, 2015
Cyathostoma (Cyathostoma) phenisci Baudet, 1937	Traqueia	Chile	González-Acuña *et al*. 2020
Família Acuariidae Railliet, Henry & Sisoff, 1912			

Syncuaria squamata (Linstow, 1883) Wong, Anderson & Bartlett, 1986	Ventrículo	Guaíba, RS	Monteiro *et al*., 2006
Superfamília Trichinelloidea Railliet, 1916 Família Capillariidae Neveu-Lemaire, 1936			
Eucoleus contortus (Creplin, 1839) Gagarin, 1951	Esôfago	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Baruscapillaria appendiculata (Freitas, 1933) Moravec, 1982	Intestino grosso e cloaca	Guaíba, RS; Pará	Monteiro, 2006; Monteiro *et al.*, 2011; Carvalho et al., 2023b
Baruscapillaria carbonis (Dubinin & Dubinina, 1940)	Intestino delgado, estômago	República Tcheca; Chile	Frantová, 2001; González-Acuña *et al*. 2020
Capillaria carbonis (Rudolphi, 1819)	Intestino delgado	Morávia do Sul, República Tcheca	Moravec, Scholz & Nasincová, 1994
Capillaria sp.	Intestino	México	Violante-González *et al*., 2011
Baruscapillaria rudolphii Moravec, Scholz et Našincová, 1994	Intestino delgado	Morávia do Sul, República Tcheca	Moravec, Scholz & Nasincová, 1994; Moravec & Scholz, 2016
Baruscapillaria spiculata (Freitas, 1933) Moravec, 1982	Cloaca	Argentina	Garbin *et al*., 2021
Baruscapillaria kamanae Presswell & Bennett, 2022	Intestino	Nova Zelândia	Presswell & Bennett, 2022
Superfamília Habronematoidea Railliet & Henry, 1915 Família Habronematidae Chitwood e Wehr, 1932			
Tetrameres gynaecophila Molin, 1859	Proventrículo, intestino	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Superfamília Acuarioidea Railliet, Henry & Sisoff, 1912 Família Acuariidae Railliet, Henry & Sisoff, 1912			
Syncuaria squamata (Linstow, 1883) Wong, Anderson & Bartlett, 1986	Proventrículo, estômago	Guaíba, RS Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Monteiro, 2006; Monteiro *et al*., 2011; Moravec & Scholz, 2016

Superfamília Dracunculoidea **Famíla Dracunculidae Stiles, 1907**			
Avioserpens Wehr & Chitwood, 1934	Estômago	Chile	González-Acuña *et al*., 2020
Filo Platyhelminthes, Classe Trematoda **Família Clinostomidae Lühe, 1901**			
Clinostomum sp.	Esôfago	Guaíba, RS	Monteiro., 2006; Monteiro *et al*., 2011
Clinostomum complanatum Rudolphi, 1814	Estômago Cavidade oral	México Japão	Violante-González *et al*., 2011; Abro *et al*., 2016a; Iwaki *et al*., 2018
Clinostomum awadhi Abro, Dharejo, Khan & Birmani, 2016	Esôfago e moela	Paquistão	Abro *et al*., 2016a
Clinostomum poteae Rosser, Baumgartner, Alberson, Noto, Woodyard, King, Wise & Griffin, 2018	Traqueia	Estados Unidos	Rosser *et al*., 2018
Diplostomum (Austrodiplostomum) compactum Lutz, 1928	Cloaca, intestino	México	Violante-González *et al*., 2011
Euclinostomum heterostomum (Rudolphi, 1809)	Esôfago	Paquistão	Abro *et al*., 2016c
Superfamília Diplostomoidea Poirier, 1886 **Família Diplostomidae Poirier, 1886**			
Hysteromorpha triloba (Rudolphi, 1819) Lutz, 1931	Duodeno, jejuno-íleo e Intestino	Guaíba, RS; Boêmia do Sul e Morávia do Sul, República Tcheca; Chile	Monteiro, 2006; Monteiro *et al*., 2011; Moravec & Scholz, 2016; González-Acuña *et al*. 2020
Austrodiplostomum mordax Szidat & Nani, 1951	Jejuno-íleo, cecos, intestino grosso e cloaca.	Guaíba, RS	Monteiro, 2006; Monteiro *et al*., 2011
Austrodiplostomum compactum (Lutz, 1928) Dubois, 1970	Jejuno-íleo, intestino grosso e cloaca.	Guaíba, RS	Monteiro, 2006; Monteiro *et al*., 2011
Superfamília Echinostomatoidea Looss, 1899 **Família Echinostomatidae (Looss, 1902) Poche, 1926**			

Drepanocephalus spathans Dietz, 1909	Jejuno-íleo e intestino grosso	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Drepanocephalus olivaceus Nasir & Marval, 1968	Jejuno-íleo, intestino	Guaíba, RS; México	Monteiro, 2006; Monteiro *et al*., 2011; Violante-González *et al*., 2011
Paryphostomum segregatum Dietz, 1909	Duodeno, jejuno-íleo e intestino grosso	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Paryphostomum sanghari Abro, Dharejo, Khan, Birmani & Naz, 2016			Abro *et al*., 2016b
Petasiger radiatus (Dujardin, 1845) Tkach, Kudlai & Kostadinova, 2015	Intestino	Boêmia do Sul e Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Petasiger exaeretus Dietz, 1909	Intestino delgado	Paquistão; Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Abro *et al*.,2016b; Moravec & Scholz, 2016
Petasiger phalacrocoracis (Yamaguti, 1939)	Intestino delgado	Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Ignavia olivacei Ostrowski de Núñez, 1967	Ureteres	Guaíba, RS	Monteiro, 2006; Monteiro *et al.*, 2011
Echinochasmus leopoldinae Scholz, Ditrich & Vargas-Vázquez, 1996	Intestino	México	Violante-González *et al*., 2011
Família Psilostomidae Looss, 1900			
Ribeiroia ondatrae (Price, 1931) Price, 1942	Proventrículo, intestino, estômago	Guaíba, RS; México	Monteiro, 2006; Monteiro *et al*., 2011; Violante-González *et al*., 2011
Família Prosthogonimidae (Lühe, 1909) Lahille, 1922			
Prosthogonimus ovatus (Rudolphi, 1803) Lühe, 1899	Cloaca	Guaíba, RS	Monteiro, 2006; Monteiro *et al*., 2007; Monteiro *et al*., 2011
Família Heterophyidae Leiper, 1909			

Cercarioides aharonii Witenberg, 1929	cloaca	Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Apophallus muehlingi (Jägerskiöld, 1899)	Intestino delgado	Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Ascocotyle (Phagicola) longa Ransom, 1920	Intestino delgado	México Morávia do Sul, República Tcheca	Violante-González *et al*., 2011; Moravec & Scholz, 2016
Ascocotyle felippei Travassos, 1929	Intestino	Chile	González-Acuña *et al*. 2020
Galactosomum lacteum (Jägerskiöld, 1896)	Intestino delgado	Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Heterophyes aequalis Looss, 1902	Intestino delgado	Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Euhaplorchis californiensis Martín 1950	Estômago	México	Violante-González *et al*., 2011
Família Opisthorchiidae (Looss, 1899) Braun, 1901			
Metorchis xanthosomus (Creplin, 1846)	Vesícula biliar	Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Família Cyathocotylidae Mühling, (1898)			
Holostephanus dubinini Vojtek et Votková, 1968	Intestino	Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Família Microphallidae Ward, (1901)			
Odhneria raminellae Travassos, 1921	Estômago	México	Violante-González *et al*., 2011
Filo Platyhelminthes, Classe Cestoda Família Dilepididae Railliet & Henry, 1909			

Paradilepis caballeroi Rysavy & Macko, 1971	Duodeno, jejuno-íleo, intestino	Guaíba, RS; México; Chile	Monteiro, 2006; Monteiro *et al*., 2011; Violante-González *et al*., 2011; González-Acuña *et al*. 2020
Filo Acanthocephala Rudolphi, 1808 Classe Palaeacanthocephala Meyer, 1931 Família Polymorphidae Meyer, 1931			
Andracantha tandemtesticulata Monteiro, Amato & Amato, 2006	Intestino	Guaíba, RS	Monteiro *et al*., 2006; Monteiro *et al*., 2011
Andracantha phalacrocoracis (Yamaguti, 1939)	Intestino	Boêmia do Sul; Morávia do Sul; Chile	Moravec & Scholz, 2016; González-Acuña *et al*. 2020
Southwellina hispida (Van Cleave, 1925) Witenberg, 1932	Intestino delgado	México; Morávia do Sul	Violante-González *et al*., 2011; Moravec & Scholz, 2016
Corynosoma arctocephali Zdzitowiecki, 1984	Intestino	Chile	González-Acuña *et al*. 2020
Profilicollis altmani (Perry, 1942)	Intestino	Chile	González-Acuña *et al*. 2020
Família Diphyllobothriidae Lühe, 1910			
Ligula intestinalis (Linnaeus, 1758)	Intestino delgado	Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Moravec & Scholz, 2016
Família Gryporhynchidae Spassky & Spasskaya, 1973			
Paradilepis scolecina (Rudolphi, 1819)	-	Boêmia do Sul, República Tcheca Morávia do Sul, República Tcheca	Moravec & Scholz, 2016

Fonte: Carvalho (2023).

1.7.Objetivos

1.7.1. Objetivo geral

Caracterizar a sistemática e relação parasito - hospedeiro de *Cairina moschata domestica* e *Phalacrocorax brasilianus* da Ilha de Marajó, Pará.

1.7.2. Objetivo específico

a) Investigar e comparar a ocorrência de helmintos nessas aves, em especial os que apresentam potencial zoonótico;
b) Estabelecer os indicadores ecológicos dos helmintos na área do estudo;
c) Realizar estudos moleculares e análises filogenéticas para redescrição de espécies ou para espécies novas de parasito;
d) Realizar análise por meio da histopatologia da relação parasita-hospedeiro;
e) Contribuir com dados sobre a biodiversidade parasitária de aves no norte do Brasil.

1.8.Artigos

Com os dados obtidos nesta pesquisa, foi possível a publicação de alguns artigos (Quadro 1), e durante o período de doutorado auxiliar na elaboração de outros manuscritos (Quadro 2).

Quadro 1- Status de produção científica dos dados obtidos de *Cairina moschata domestica* e *Phalacrocorax brasilianus* no município de Soure, Ilha de Marajó, Estado do Pará, Brasil.

Título	Status	Revista	Qualis	Fator de Impacto
Lesions Caused by Anisakids and Capillariids in *Cairina moschata* raised on Marajó Island, State of Pará, Brazil	Publicado	Arquivo Brasileiro de Medicina Veterinária e Zootecnia	B1	0,40
Diversity of endohelminths parasitizing bred Muscovy Ducks *Cairina moschata domestica* (Anseriformes: Anatidae) from the Eastern Brazilian Amazon	Publicado	Journal of Parasitic Diseases	B3	1,2

A new nematode of the family Capillariidae identified in *Cairina moschata* (Linnaeus) on Marajó Island in the Brazilian Amazon	Publicado	Revista Brasileira de Parasitologia Veterinária	A2	1.3
Baruscapillaria appendiculata (Nematoda: Capillariidae) Parasite of *Phalacrocorax brasilianus* (Suliformes: Phalacrocoracidae) In Marajó Island, Pará, Brazilian Amazon	Publicado	Revista Brasileira de Parasitologia Veterinária	A2	1.3
Community of helminths parasitizing cormorants (Suliformes: Phalacrocoracidae) from the Brazilian Eastern Amazon, Pará	Em elaboração	Veterinary Parasitology: Regional Studies and Reports	A3	1.4
Singamidae of the birds in the Brazilian Amazon	Em elaboração	Parasitology Research	A2	2.0

Quadro 2- Status de produção científica ao longo do curso de doutorado no período de 2020 a 2023.

Título	**Status**	**Revista**	**Qualis**	**Fator de Impacto**
Morphological and molecular characterization of *Contracaecum australe* (Nematoda: Anisakidae) parasitizing *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) on the north coast of Brazil	Publicado	Revista Brasileira de Parasitologia Veterinária	A2	1.3
Redescription of *Brevimulticaecum baylisi* (Travassos, 1933) Sprent (1979) (Nematoda: Heterocheilidae), a parasite of *Caiman crocodilus* (Crocodylia: Alligatoridae) in the north-eastern Peruvian Amazon	Publicado	Veterinary Parasitology: Regional Studies and Reports	A3	1.4
Ozolaimus megatyphlon and *Ozolaimus cirratus* parasitizing the *Iguana iguana* (Linnaeus, 1758) from Marajó Island, Pará, Brasil: new occurrence and morphological redescription	Publicado	Revista Brasileira de Parasitologia Veterinária	A2	1.3
A molecular survey of three tick-borne pathogens in dogs from Algodoal village/Maiandeua island on the northeast coast of Pará, Brazil	Publicado	Archives of Veterinary Science	B2	0,15

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ARTIGO 1

Título: LESIONS CAUSED BY ANISAKIDS AND CAPILLARIIDS IN *Cairina moschata* RAISED ON MARAJÓ ISLAND, STATE OF PARÁ, BRAZIL

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Lesions caused by anisakid and capillariid in *Cairina moschata* raised on Marajó island, state of Pará, Brazil

[*Lesões por anisakideos e capillarideos em* Cairina moschata *criados na Ilha de Marajó, estado do Pará, Brasil*]

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ABSTRACT

The Muscovy duck is a commercially important bird on the island of Marajó usually raised in a peculiar system that includes supplying fish viscera to the birds under semi-extensive farming conditions. This enables a risk of contamination and losses in the production of these birds, resulting from injuries caused by helminth infections, especially nematodes. The objective of this study was to evaluate the histopathological changes caused by nematodes of the genera: *Eucoleus*, *Anisakis* and *Contracaecum*. Thirty-three ducks with lesions in the esophagus and ventricle were analyzed. Histopathological exams showed a mild inflammatory infiltrate in the submucosa of the esophagus caused by the fixation of *E. contortus* and third stage larvae of *Anisakis* sp., and we recorded third stage larvae of *Contracaecum* sp. parasitizing the ventricle, this being the first record of this parasite in ducks in Brazil.

Keywords: histopathology, esophagus, birds, parasitism, helminths

RESUMO

O pato doméstico é uma ave amplamente comercializada na Ilha de Marajó, com um peculiar manejo que inclui a oferta de vísceras de peixes aos animais em criações semiextensivas, propiciando, assim, risco de contaminação e perdas na produção dessas aves decorrentes de lesões oriundas de infecções por helmintos, especialmente os nematódeos. Nesse sentido, objetivou-se avaliar as alterações histopatológicas causadas por nematódeos dos gêneras: Eucoleus, Anisakis *e* Contracaecum. *Foram analisados 33 patos, e três exemplares apresentaram lesões no esôfago e no ventrículo. Exames histopatológicos demonstraram discreto infiltrado inflamatório na submucosa do esôfago ocasionado pela fixação de* E. contortus *e larvas de terceiro estágio de* Anisakis *sp., bem como foram registradas larvas de terceiro estágio de* Contracaecum *sp. parasitando o ventrículo, sendo esse o primeiro registro desse parasito em patos no Brasil.*

Palavras-chave: histopatologia, esôfago, aves, parasitismo, helmintos

INTRODUCTION

Cairina moschata ducks (Linnaeus, 1758) are bred for the production of eggs and meat for both self-consumption and for sale (Meulen and Dikken, 2003). Muscovy ducks are birds that have filtering habits and are not selective in terms of food, especially when raised extensively, where they absorb enough proteins in the environment, feeding on grass, small fish, crustaceans and insects (Sick, 1997; Meulen and Dikken, 2003). Livelihood creations are common

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in Brazil, and occur mainly among small producers, commercial houses and open markets, where the hygienic sanitary conditions of this type of creation are not clarified (Souza Almeida *et al.*, 2016). In northern Brazil they are appreciated in the cuisine of Pará and have high commercial value mainly during festive seasons.

Breeding birds under extensive or free-range systems in the environment facilitates the occurrence of endoparasites. That is a point of concern in the different systems of duck farming as it leads to economic losses, and preventive measures are considered the most effective strategy (Rennó *et al.*, 2008). The rate of helminth infection is worrisome, especially since ducks may show unspecific clinical signs of those infections (Cubas, 2007; Rosa and Shivaprasad, 2015). In addition, these helminthiasis are the main diseases that affect birds reared in an extensive regime, causing an increase in the mortality rate, as well as providing the dissemination of a wide variety of parasite species in the environment (Menezes, 1999; Gomes *et al.*, 2009). Among the main changes in the host organism caused by the parasites are spoliation and the inflammatory process resulting from the process of migration, fixation or type of food performed by the parasite, which can vary with the degree of intensity of infection (Menezes *et al.*, 2001; Neves, 2016).

Menezes *et al.* (2001) described macro and microscopic changes found in several organs, among them *Numida meleagris* Linnaeus, 1758 esophagus and crop parasitized by *Eucoleus perforans*, of the macroscopic changes the authors observed that the parasites caused petechiae and congestion, and microscopy showed the parasites inserted deep in the stratified squamous epithelium of the crop, with intense inflammatory reaction and distension of the mucous glands present in the crop's own tunic. These changes by the parasite were considered serious, even with low average intensity of infection. Data on helminths in ducks are scarce and little known, particularly about the spoliative action of the presence of parasites and their relationship with host tissue (Mattos Junior *et al.*, 2008). The investigation of the biota of helminths of Muscovy ducks on the Island of Marajó, can help in the sanitary control and in the productivity of these animals. This study thus aimed to describe the histopathological aspects of infection and lesions in the tubular digestive system of *Cairina moschata*, caused by *Eucoleus contortus* (Capillariidae), *Anisakis* sp. (Anisakidae) and *Contracaecum* sp. (Anisakidae), in order to contribute to the medical clinic and sanitary management of this species, of this bird, raised free on the Island of Marajó.

MATERIAL AND METHODS

Thirty-three specimens of *C. moschata* were purchased from rural properties in the municipality of Soure (00º 43' 00" S; 48º 31' 24" W), Marajó Island, State of Pará, under protocols of the Ethics Committee on the use of animals (CEUA) No. 030/2018 and the Federal Rural University of the Amazon (UFRA) No. 23084.014807 / 2018-80. They were necropsied in the laboratory to search for helminths, where each organ was carefully analyzed with the aid of the Leica ES2 stereomicroscope, and three specimens presented nematodes fixed to the esophagus and ventricles, from which fragments were removed and fixed in 10% formalin, and processed according to routine histological techniques (Tolosa *et al.*, 2003). Photomicrographs of the slides were captured and analyzed using a Leica DM2500 microscope with an attached digital camera.

The nematodes collected were fixed in A.F.A (93 parts of 70% ethyl alcohol, 5 parts of formaldehyde and 2 parts of glacial acetic acid), overnight, transferred to a solution containing 70% ethanol. For taxonomic identification, the nematodes specimens were clarified with 20% Aman lactophenol and temporarily mounted between slides and coverslips for observation of morphological characters under a LEICA DM2500 light microscope with an imaging capture system. For the taxonomic classification of nematodes, the works of Vicente *et al.* (1995), Moravec (1998), De Ley and Blaxter (2002), Felizardo *et al.* (2009), Gibbons (2010) and Fonseca *et al.* (2016) were consulted. To determine the ecological indexes of parasitism, these helminths will be analyzed by means of prevalence (%), average intensity of infection (IMI) and average abundance, according to Bush *et al.* (1997).

RESULTS

The nematodes recovered were found inserted in the esophagus and ventricle of muscovy duck in Marajó Island and morphologically identified as third stage larvae *Anisakis* (Anisakidae) (Figure 1A), and adults from *Eucoleus contortus* (Capillariidae) were also found parasitizing the esophagus with a prevalence of 9.1% and 75.8% respectively. Third stage larvae of *Contracaecum* (Anisakidae) were found inserted in the ventricle in 12.1% of the ducks. The parasitological indices of these nematodes in *Cairina moschata* are shown in Table 1.

Table 1. Parasitological indices of Capillariidae and Anisakidae in *Cairina moschata* (n = 33) from the eastern Amazon (Brazil)

SI	Parameters	*Eucoleus contortus*	*Anisakis* sp.	*Contracaecum* sp.
Esophagus	P (%)	75.8	9.1	3.0
	MI	11.2	95.7	3.0
	MA	85	8.7	0.1
	TNP	281	287	3
Gizzard	P (%)	9.1	9.1	0
	MI	10.7	0.3	0
	MA	1	0.03	0
	TNP	32	1	0
Proventriculus	P (%)	12.1	0	6.1
	MI	17.3	0	3.5
	MA	2.1	0	0.2
	TNP	69	0	7
Ventriculus	P (%)	0	0	12.1
	MI	0	0	2.3
	MA	0	0	0.3
	TNP	0	0	9
Intestine	P (%)	0	3.0	3.0
	MI	0	1.0	2.0
	MA	0	0.03	0.1
	TNP	0	1	2

SI: infection site, P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites.

Histopathological analysis of infection by *Anisakis* sp. in the esophagus of *Cairina moschata*, showed that the lesion is predominantly in the mucosa, and able to transpose the muscle of the mucosa and affect the internal muscular layer. The stratified squamous epithelium was found to be intact. However, in the parasite-host relationship, alterations are present in the mucous glands due to the location of the nematodes, which promotes glandular destruction and tissue reaction with accumulations of cellular debris at the tissue-parasite interface, bordered by a foreign body-type granulomatous reaction due to the presence of giant cells (Figure 1B). The lamina propria with prominent and variable infiltration of eosinophils in addition to lymphocytes and staining by Gomori Trichrome did not show fibroplasia associated with inflammation. The connective tissue adjacent to the compromised gland was looser, probably due to tissue edema. In the cross section of the parasite *Anisakis*, it was possible to observe the epidermal cord in a "Y" shape (Figure 1C).

Histologically, the infection of the ventricle (gizzard) by *Contracaecum* sp. demonstrates that the nematode is fixed in the mucosa, below the glycoprotein secretion layer, where no tissue reaction due to the presence of the parasite was observed (Figure 1D). In the macroscopic image, we can observe the fixation of the parasite in the ventricle (Figure 1E). Esophageal infection by *Eucoleus contortus* showed pseudo-encapsulation of the parasite in the mucosal pavement epithelium, with mild compressive atrophy of keratinocytes. There was also mild exocytosis due to the presence of eosinophils among the keratinocytes and in the submucosa the inflammatory infiltrate of eosinophils is mild and variable (Figure 1F).

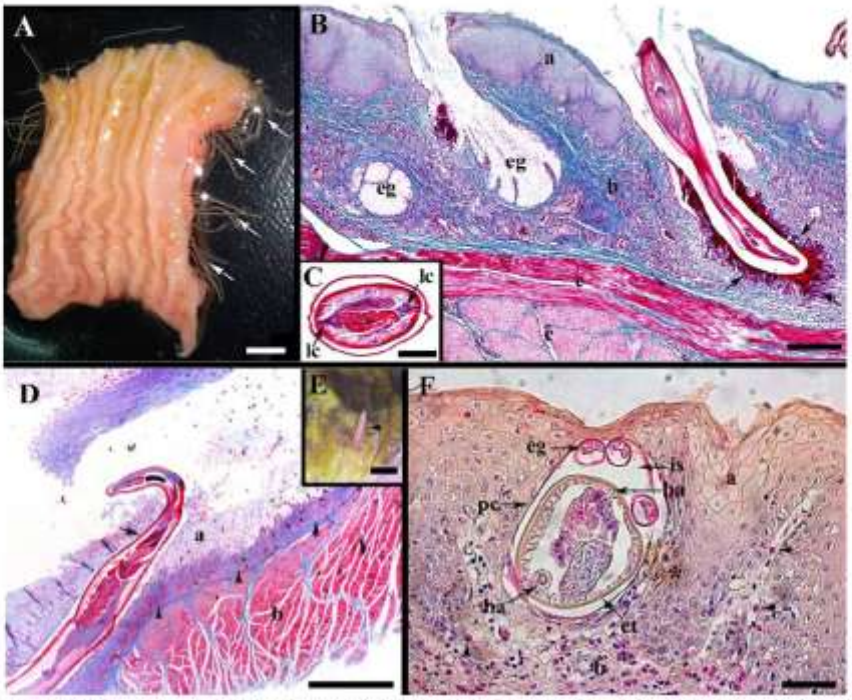

Figure 1. (A) Mesoscopic view of *Anisakis* sp. (arrowhead) inserted in the esophagus of a Muscovy duck on Marajó Island, State of Pará; (B) Photomicrographs of the esophagus parasitized by *Anisakis* sp. larvae (arrows) stained with Gomori Trichrome: a = stratified squamous epithelial tissue, of the esophageal lining, b = subepithelial connective tissue, c = smooth muscle tissue and eg = esophageal gland; (C) Detail of the cross section of an *Anisakis* sp. larva, showing lateral epidermal cord (arrow) and intestine (*). Gomori Trichrome; (D) Photomicrographs of the ventricle parasitized by *Contracaecum* sp. in Muscovy duck on Marajó Island stained with Gomori Trichrome: a = mucous layer of the ventricle, tubular glands of the ventricle (arrowhead) and b = muscular layer of the ventricle, showing the well-developed smooth muscle layer; (E) Mesoscopic view of a *Contracaecum* sp. (arrowhead) inserted in the Muscovy duck ventricle; (F) Photomicrographs of the esophagus parasitized by a female *Eucoleus contortus* in a Muscovy duck on Marajó Island stained with hematoxylin and eosin: a = stratified squamous epithelial tissue of the lining of the esophagus mucosa, b = loose connective tissue of the submucosa with abundant eosinophils (arrowhead), detail of the pseudo-capsule (pc) surrounding the parasite, forming an interstitial space (s) containing eggs (eg), and the nematode with the presence of a thick cuticle (ct) and the presence of bacillary bands (ba), compressive atrophy of keratinocytes (*) of the stratified squamous epithelial tissue of the esophagus. Scale bars: A = 2 cm, B = 200 µm, C = 100 µm, D = 1 mm, E = 2 cm; F: 50 µm.

DISCUSSION

In Brazil, Mattos Junior *et al.* (2008), detected in *Cairina moschata* of Rio de Janeiro a parasitic biota of capillariids nematodes composed of 20% of *Capillaria phasianina* Kotlán, 1914 in esophagus and caecum; 30% of *Capillaria* sp. Pinto and Almeida, 1935 in esophagus, cecum and gallbladder, and 6.6% of *Eucoleus cairinae* (Freitas and Almeida, 1935) Lopez and Neyra, 1947 in esophagus, and histopathological studies of organs injured by parasitism have not yet been published. In our study, the major sites of infection were esophagus with *Eucoleus contortus* and *Anisakis* sp. and the ventricle with *Contracaecum* sp., these sites showed lesions by the parasites, and after histopathological processing it was possible to observe the changes caused by capillary and anisakid nematodes in the tissues.

Histopathological changes caused by the presence of *Eucoleus contortus*, *Anisakis* sp., *Contracaecum* sp., are recorded herein for the first time in our research parasitizing the esophagus and ventricle of Muscovy duck in Brazil. These birds raised without confinement, look for food in areas of rivers or lakes, with no selectivity in feeding (Meulen and Dikken, 2003), which may justify the presence of these anisakid and capillary nematodes in birds (Carvalho *et al*., 2019, 2020). Different studies have been carried out in order to characterize the parasitic fauna of ducks around the world. Among them Bano *et al*. (2005) recorded the presence of *Streptocara incognita* Gibson, 1968 in the esophagus of ducks in Italy. In the same host, Muhairwa *et al*. (2007) recorded *E. contortus*, *E. annulatus* (Molin, 1858) and *Capillaria anatis* (Schrank, 1790) parasitizing the intestinal caecum. Likewise, Yousuf *et al*. (2009), when assessing the fauna present in the gastrointestinal tract of ducks, registered the presence of *Amidostomum anseris* and *E. contortus*.

The presence of *E. contortus* and third stage larvae of *Anisakis* sp. inserted in the esophageal tissues of Muscovy ducks induced an inflammatory reaction in the tissue of this organ, as well as the migration of defense cells to this region, especially to the site of larval fixation, caused intense inflammatory infiltrate resulting from foreign body reaction and edema. Railliet and Lucet (1889) described the pathogenic effects by means of hispathological analysis in the gall bladder and esophagus of Galliformes and Anseriformes parasitized by *E. contortus*, thus demonstrating a major inflammatory process. According to Cram (1936), the Galliformes analyzed in his research were infected with *E. contortus* eggs and after death revealed necrosis of the esophageal epithelium, delicate connective tissue capsules that surrounded the areas containing the parasites and their eggs, tissue with lymphocytes and large mononuclear leukocytes, with some lesions extended to the submucosa. Pinto *et al*. (2004) observed lesions by the parasite *Eucoleus perforans* in the esophagus of *Phasianus colchicus*, where the parasites were deeply inserted in the stratified squamous epithelium, without inflammatory reaction, there was also thickening and papillary transformation of the mucosa.

Several third-stage larvae of *Anisakis* sp. were found strongly inserted in the esophagus of Muscovy ducks, causing an intense inflammatory infiltrate, resulting from a foreign body reaction and edema. According to Junqueira (2018), parasites that affect the crop and esophagus are especially harmful, as they can severely damage the mucosa of the affected organ, inducing edema and thickening of the mucous membranes, making eating difficult and even preventing birds from eating food. In cross section of the esophagus of the birds in our study, it was possible to observe the larvae of *Anisakis* sp. and their identifying structures. Murata *et al*. (2018) and Lauwers *et al*. (2017), who recorded the occurrence of anisakiasis in humans, was found as a definition of gender, found the shape of the lateral epidermal cord and the intestinal lumen in "Y" to be determinants for *Anisakis* spp. Kim *et al*. (2006) during histopathological analysis in humans recorded a lesion in the submucosa of the stomach that showed severe eosinophilic infiltrations and inflammation.

Anisakids, in their larval and adult forms frequently present important pathological lesions in the alimentary tract and associated organs in their natural host species (Smith, 1999). According to Lymbery and Cheah (2007), the larvae of these nematodes also promote a series of pathological effects in accidental hosts, such as humans. This reinforces the need for attention and care in proper management during the removal and disposal of the viscera of ducks infected with L3 larvae of anisakids, avoiding the migration of these larvae to the musculature of the bird in the postmortem and the possible risks to human health due to ingestion of contaminated meats (Carvalho *et al*., 2020). In this study, the presence of *Contracaecum* sp. in the ventricle did not produce injuries in that organ. In contrast, Vicente *et al*. (1995) and Amato *et al*. (2006), registered the presence of different larval stages of *Contracaecum* sp. parasitizing the proventriculus and ventricles of birds of the orders Falconiformes, Accipitriformes, Pelecaniformes, Suliformes, Ciconiiformes, Sphenisciformes, inducing the formation ulcerated eosinophilic granulomas.

Among the parasites that present risks to human health, nematodes of the Anisakidae family can be highlighted. Their intermediate hosts are fish cephalopod molluscs, and small crustaceans

(Adams *et al*., 1997), all animals that can compose the diet of ducks raised free in the wild. In Brazil, investigations related to helminth-induced diseases have been described in other bird species such as *Phasianus colchicus* and *Meleagris gallopavo* by Pinto *et al*. (2004, 2008). In terms of pathogenesis, when there is a major infection it can be considered extremely harmful to birds (Pizarro *et al*., 2000). The clinical signs presented by Muscovy ducks can be nonspecific (Rosa and Shivaprasad, 2015), meaning that helminthiasis can lead to an economic problem, as well as to the health of domestic poultry breeding systems (Cubas, 2007). Therefore, preventive health care such as good hygiene, deworming and vaccination of birds can reduce the rate of emergence of various parasites and other diseases (Meulen and Dikken, 2003).

CONCLUSION

The Muscovy duck is the new host of *Contracaecum* sp. in the state of Pará, being important, as there were still no studies on this parasite in this bird. And we also added the description of histopathological changes, such as intense inflammatory reactions in the esophagus caused by *Anisakis* sp. and *Eucoleus contortus*, and the presence of *Contracaecum* sp. in the ventricle, thus contributing to the knowledge of the pathogenicity of these parasites in *Cairina moschata* created and consumed by the island's population.

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ARTIGO 2

Título: DIVERSITY OF ENDOHELMINTHS PARASITIZING BRED MUSCOVY DUCKS *Cairina moschata domestica* (ANSERIFORMES: ANATIDAE) FROM THE EASTERN BRAZILIAN AMAZON

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ORIGINAL ARTICLE

Diversity of endohelminths parasitizing bred Muscovy ducks *Cairina moschata domestica* (Anseriformes: Anatidae) from the eastern Brazilian Amazon

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Abstract Raising of Muscovy ducks *Cairina moschata domestica* for subsistence of human populations is common in northern Brazil, although their helminth infections have been poorly investigated, despite the possible presence of helminths with zoonotic potential. The aim of this study was to investigate the diversity of parasite endohelminths in *C. moschata domestica* raised in the Marajó Island region, state of Pará, Brazilian Amazon region. Of 33 specimens examined, 90.9% were parasitized by one or more parasite species, for a total of 926 parasites recorded. The species mean richness of endohelminths varied from 0 to 6, and there was a predominance of hosts with 1 to 2 species of parasite endohelminths and low prevalence and low abundance of parasites. This was the first report of larvae of *Anisakis* sp., *Contracaecum* sp., *Hysterotylacium* sp., *Raphidascaris* sp., *Eustrongylides* sp., *Syngamus* sp., *Ascocotyle* sp. and *Athesmia heterolecithodes* for *C. moschata domestica*. The parasitic community of *C. moschata domestica* was composed of 11 species, with a high species richness for nematode species and a small number of digeneans.

Keywords Avian · Diversity · Helminths · Infection · Parasites

Introduction

Muscovy ducks *Cairina moschata domestica* (Linnaeus, 1758) are birds adapted to varied climatic conditions and have been widely bred due to their ease in handling (Béjcek & Stastný 2008). They have adapted to the captive breeding system, especially when that involves cool places with good availability of water and space (Geromel 2012). In Brazil, ducks for subsistence of human populations are common, but the hygienic and sanitary conditions of raising have been little investigated (Souza-Almeida et al. 2016). Although ducks have great physical resistance, they are susceptible to disease because they are particularly prone to infections caused by parasitic helminths (Gower 1939; Mattos-Junior et al. 2008). However, the helminth infections of the Muskovy ducks *C. moschata domestica* have been poorly studied (Carvalho et al. 2019), despite possible losses in terms of reduced body growth and mortality in breeding, as well as the presence of helminths with zoonotic potential. Carvalho et al. (2020) described the first report of *Anisakis* sp. parasitizing Muscovy duck in Marajó Island, State of Pará (Brazil). In addition, *Gnathostoma* sp. has been reported in tetraodontiform

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Colomesus Psittacus (Bloch & Schneider 1801) from Marajó Island (Pinheiro et al. 2017).

Infection by nematodes, cestodes and trematodes has been reported for *C. moschata domestica* (Table 1), although only a few studies have been carried out with

Table 1 Endohelminths in *Cairina moschata domestica* from different localities

Parasite species	Taxon	Locality	References
Eucoleus cairinae Lopez-Neyra, 1947	Nematoda	Brazil	Vicente et al. (1995)
Eucoleus contortus Gagarin, 1951	Nematoda	Brazil	Carvalho et al. (2019)
Eucoleus contortus Creplin, 1839	Nematoda	Tanzania	Muhairwa et al. (2007)
Heterakis gallinarum Schrank, 1788	Nematoda	Brazil	Vicente et al. (1995)
Heterakis gallinarum Schrank, 1788	Nematoda	Brazil	Machado et al. (2006)
Heterakis gallinarum Schrank, 1788	Nematoda	Tanzania	Muhairwa et al. (2007)
Heterakis neglecta Chabaud, 1975	Nematoda	Brazil	Vicente et al. (1995)
Heterakis neglecta Chabaud, 1975	Nematoda	Brazil	Machado et al. (2006)
Tetrameres fissispina Travassos, 1914	Nematoda	Brazil	Vicente et al. (1995)
Hadjelia neglecta Chabaud, 1975	Nematoda	Brazil	Mattos-Junior et al. (2008)
Capillaria phasianina Kotlán, 1914	Nematoda	Brazil	Mattos-Junior et al. (2008)
Tetrameres fissispina Travassos, 1914	Nematoda	Brazil	Mattos-Junior et al. (2008)
Eucoleus cairinae Lopez & Neyra, 1947	Nematoda	Brazil	Mattos-Junior et al. (2008)
Ascaridia columbae Gmelin, 1790	Nematoda	Tanzania	Muhairwa et al. (2007)
Ascaridia dissimilis Pérez-Vigueras, 1931	Nematoda	Tanzania	Muhairwa et al. (2007)
Ascaridia galli Schrank, 1788	Nematoda	Tanzania	Muhairwa et al. (2007)
Capillaria anatis Schrank, 1790	Nematoda	Tanzania	Muhairwa et al. (2007)
Capillaria annulata Molin, 1858	Nematoda	Tanzania	Muhairwa et al. (2007)
Heterakis dispar Schrank, 1790	Nematoda	Tanzania	Muhairwa et al. (2007)
Heterakis isolanche Linstow, 1906	Nematoda	Tanzania	Muhairwa et al. (2007)
Subulura strongylina Rudolphi, 1819	Nematoda	Tanzania	Muhairwa et al. (2007)
Subulura brumpti Cram, 1927	Nematoda	Tanzania	Muhairwa et al. (2007)
Subulura suctoria Molin, 1860	Nematoda	Tanzania	Muhairwa et al. (2007)
Tetrameres sp.	Nematoda	Brazil	Vicente et al. (1995)
Tetrameres sp.	Nematoda	Brazil	Machado et al. (2006)
Heterakis sp.	Nematoda	Brazil	Vicente et al. (1995)
Heterakis sp.	Nematoda	Brazil	Machado et al. (2006)
Subulura sp.	Nematoda	Brazil	Vicente et al. (1995)
Subulura sp.	Nematoda	Brazil	Machado et al. (2006)
Capillaria sp.	Nematoda	Brazil	Vicente et al. (1995)
Capillaria sp.	Nematoda	Brazil	Mattos-Junior et al. (2008)
Anisakis sp.	Nematoda	Brazil	Carvalho et al. (2020)
Raillietina echinobothrida Mégnin, 1880	Cestoda	Tanzania	Muhairwa et al. (2007)
Raillietina tetragona Molin, 1858	Cestoda	Tanzania	Muhairwa et al. (2007)
Fimbriaria fasciolaris Frolich, 1802	Cestoda	Brazil	Mattos-Junior et al. (2008)
Lateriporus sp.	Cestoda	Brazil	Mattos-Junior et al. (2008)
Prosthogonimus sp.	Trematoda	Brazil	Machado et al. (2006)
Echinostoma revolutum Frolich, 1802	Trematoda	Brazil	Mattos-Junior et al. (2008)
Echinostoma mendax Dietz, 1909	Trematoda	Brazil	Machado et al. (2006)
Echinostoma revolutum Froelich, 1802	Trematoda	Brazil	Machado et al. (2006)
Ophthalmophagus magalhaesi Travassos, 1921	Trematoda	Brazil	Machado et al. (2006)
Zigocotyle lunatum Diesing, 1836	Trematoda	Brazil	Machado et al. (2006)
Typhlocoelum cucumerinum Rudolphi, 1809	Trematoda	Brazil	Machado et al. (2006)

domestic animals (Muhairwa et al. 2007; Mattos-Junior et al. 2008; Carvalho et al. 2019, 2020). Muscovy ducks are one of the food items for human populations in the northern region of Brazil, including Marajó Island, state of Pará, in the Brazilian Amazon. Eggs of nematodes, trematodes and cestodes were reported from *Cyanoloxia rothschildii*, *Paroaria gularis* and *Tangara episcopus* kept in captivity in the city of Belém, State of Pará (Magalhães-Matos et al. 2016). As there are few studies about helminths in *C. moschata domestica*, the knowledge related to diversity of these parasites is consequently limited, and the community structure of endohelminths is unknown so far.

Several patterns associated with parasitic communities of different animal host populations can be detected through quantitative descriptors as diversity index and species richness (Magurran 2004). Such studies provide relevant information about avian host populations and expand the knowledge of parasite-host-environment interactions (Bush et al. 1997; Sanmartín et al. 2004; Carvalho et al. 2020). Since several studies in bird hosts (Gower 1939; Alexander & Mclaughlin 1997; Sanmartín et al. 2004; Junker et al. 2008), have focused on the determinant factors structuring the species richness of the communities of parasitic helminths, a diverse and interactive community can be expected, based on their complex habitats and feeding practices (Alexander & Mclaughlin 1997; Navarro et al. 2005). The local populations of potential definitive and intermediate hosts also have an important influence on helminth community patterns in avian species because of the set of helminths they support (Alexander & Mclaughlin 1997; Navarro et al. 2005; Carvalho et al. 2020). Thus, the aim of this study was to investigate the diversity of parasite endohelminths in *C. moschata domestica,* raised in the region of Marajó Island, state of Pará (Brazil).

Materials and methods

Ducks and collection

Collection was performed from June 2018 to August 2019 in accordance with the Ethics Committee for Animal Use (CEUA-UFRA) under protocol N° 030/2018. Thirty-three *C. moschata domestica* ducks (21 females and 12 males, aged 4–8 months) were acquired from rural properties of the municipality of Soure (00° 43′ 00″ S; 48° 31′ 24″ W), on Marajó Island. The ducks were components of small extensively raised herds with free access to the environment, used to provide food for families or for sale in local markets. The ducks were slaughtered by stunning with a club, cutting the blood vessels of the neck and exsanguination on the farm and only the organs of the digestive tract were transported to the Laboratório de Histologia e Embriologia Animal/UFRA, Campus Belém (PA, Brazil) for parasitological analyses. The organs are placed in air-tight bags, identified and transported in an isothermal box containing ice. In the laboratory, all the duck parts were weighed in a balance (Ramuzatron 15 BAT, Brazil), and organs were separated, individualized, and placed in Petri dishes with NaCl 0.9% solution and examined in less than 24 h, using a stereomicroscope (LEICA-ES2) using ampliation from 5 x (Carvalho et al. 2019). The contents of the lumen and wall were individualized in Petri dish and analyzed separately using a stereomicroscope (LEICA-ES2) using ampliation from 5 x. We do not use sieves because the content was analyzed separately with the aid of a stereomicroscope (LEICA-ES2), being only removed from the analysis of corn grain and plant fragments. The lining of the gizzard was removed to search for parasites. The recovered nematodes were fixed in a solution of AFA (93 parts of 70% ethanol, 5 parts of formaldehyde, and 2 parts of glacial acetic acid) and processed using light microscopy and scanning electron microscopy according to the method described by Pinheiro et al. (2018). Amman's lactophenol (crystallized phenol 20 g, lactic acid 20 mL, glycerin 40 mL and distilled water 20 mL) was used to observe the internal structures of nematodes for taxonomic identification. Nematodes were clarified with Amman's lactophenol solution, placed on a microscope slide under a coverslip as a temporary mount, observed using a light microscope, and photographed using a photomicroscope (LEICA DM2500) with an imaging capture system. The trematodes specimens were compressed and fixed in AFA, stained with alcoholic carmine, dehydrated in an alcohol series, clarified in methyl salicylate and mounted on permanent slides for light microscopy analysis. Taxonomic classification of nematodes was according to Moravec (1982); Vicente et al. (1995) and Gibbons (2010), and for trematodes was based in the works of Travassos (1969); Jones et al. (2002); Jones et al. (2005) and Bray et al. (2008).

Parasite collection and analysis

Ecological terms (prevalence, mean intensity and mean abundance) were used following Bush et al. (1997). We used Diversity software (Pisces Conservation Ltd., UK) to calculate the following descriptors for the parasite community: for species richness of parasites, the Brillouin diversity index (*HB*), evenness (*E*) in association with the diversity index, and the dominance frequency (percentage of the infrapopulation in which a parasite species is numerically dominant) (Rohde et al. 1995; Magurran 2004). In order to detect the distribution pattern of the parasite infrapopulation (Rózsa et al. 2000), the index of

dispersion (ID) and the Poulin discrepancy index (D) were calculated using Quantitative Parasitology 3.0 software for species with prevalence > 10%. The ID significance for each infrapopulation was tested using *d*-statistics (Ludwig & Reynolds 1988).

The Shapiro–Wilk test was applied to determine whether the parasite abundance data followed a normal distribution, and Barlett test to homocedasticity. The G-test was used for determining the sex effect of host sex in the prevalence of parasites, and the Mann–Whitney test (*U*), was employed to compare the abundance of species of parasites between male and female hosts. The Spearman correlation coefficient (*rs*) was used for evaluating possible correlation of abundance and prevalence of helminths with the weight of hosts, as well as with the Brillouin index (Zar 2010).

Results

Of 33 specimens of *C. moschata domestica* examined, 90.9% were parasitized by one or more parasite species, and a total of 926 parasites were collected from different organs. A predominance of nematode larvae such as *Anisakis* sp., *Contracaecum* sp., *Hysterotylacium* sp., *Raphidascaris* sp., *Eustrongylides* sp., *Syngamus* sp., *Capillaria* sp. and *Subulura* sp. was found. Among these parasites, *Anisakis* sp. and *Eucoleus contortus* Creplin, 1839 were the dominant species (Table 2). Infection by *Anisakis* sp. presented a random dispersion, while *Capillaria* sp. and *E. contortus* presented an aggregated dispersion (Table 3).

No difference (G = 1.25, $p = 0.26$) in prevalence (P) of endohelminth parasites between females (P = 95.2%, n = 21) and males (P = 83.3%, n = 12) was found, as well as (U = 84.00, $p = 0.09$) between the mean abundance of parasites (MA) in females (MA = 38.5 ± 74.9) and males (MA = 11.9 ± 14.7). No correlation ($rs = -0.270$, $p = 0.128$) of endohelminth abundance with the weight of hosts and with the Brillouin index ($rs = -0.234$, $p = 0.188$) was found. No correlation ($rs = 0.279$, $p = 0.124$) of endohelminth prevalence with the weight of hosts) was found.

The species mean richness of endohelminths varied from 0 to 6, Brillouin diversity index from 0 to 1.2 and evenness from 0 to 0.5 (Table 4).

Discussion

The fauna of endohelminths in *C. moschata domestica* was composed of 9 species of Nematoda and two species of Trematoda found in different organs, and was characterized by a predominance of nematode larvae, low diversity, low species richness and low evenness. Mattos-Junior et al. (2008) reported seven species of endohelminths for domestic *C. moschata domesticus* in Rio Janeiro State (Brazil), five species of Nematoda, one Cestoda and one Trematoda. For free range *C. moschata* in Tanzania, the endohelminth fauna was constituted by 12 species of Nematoda and two species of Cestoda (Muhairwa et al. 2007) (Table 1). Although these studies did not provide information on the stomach contents of this avian host, they are known to ingest a certain range of prey in their habitats, thus exposing themselves to potential intermediate hosts, which accounts for this distinctly differentiated fauna of parasitic helminths. Thus, in addition to dietary differences being a significant factor in defining the helminth fauna in these host populations, spatial factors may also have played an important role in these hosts.

Among wild avian species, it is suggested that such hosts harbor a richer diversity of helminths (Santoro et al. 2012) than domestic birds, because the fauna and species richness of parasite helminths are influenced by specific factors such as particular habitat, geographic range, a broad host diet and selective feeding by the host on prey that serve as intermediate hosts (Alexander & Mclaughlin 1997; Sanmartín et al. 2004; Junker et al. 2008; Santoro et al. 2012). Therefore, the specimens of *C. moschata domestica* examined in our study had fed selectively on prey species that are intermediate hosts of endohelminths, because they acquired a richer diversity of nematode larvae species than trematoides.

In avian species, factors governing the dynamics of spatial distribution of endohelminth species are varied and complex. The most dispersion common pattern of parasites is the aggregated distribution (Sanmartín et al. 2004; Junker et al. 2008), which indicates that the parasite species has achieved a certain degree of stability. This pattern of dispersion can be due to the wide dimensions of the hosts' ecological niches, the genetic heterogeneity and the heterogeneity of exposure and susceptibility of the host population (Dobson 1990; Poulin, 2013). In contrast, the random dispersion pattern is characteristic of parasite populations in the early stages of colonization of a new environment or in decline, which thus show low population densities (Sanmartín et al. 2004). Therefore, an aggregated dispersion pattern depends on factors such as genetic heterogeneity, exposure and susceptibility of the host population, feeding preferences and species-specific host behavior and environmental factors (Sanmartín et al. 2004; Junker et al. 2008). In *C. moschata domestica*, we found that larvae of *Anisakis* sp., a moderately abundant endohelminth, had a random dispersion, while *E. contortus* and larvae of *Capillaria* sp. had aggregated dispersion. Those parasite species have different niches within the host and are thus not in direct competition. In parasite species that

Table 2 Parasitological indices of endohelminths in *Cairina moschata domestica* (N = 33) from the eastern Amazon (Brazil)

SI	Parameters	*Eucoleus contortus*	*Anisakis* sp.	*Contracaecum* sp.	*Hysterotylacium* sp.	*Raphidascaris* sp.	*Eustrongylides* sp.	*Syngamus* sp.	*Capillaria* sp.	*Subulura* sp.	*Athesmia heterolecithodes*	*Ascocotyle* sp.
Esophagus	P (%)	75.8	9.1	3.0	6.1	3.0	0	0	0	0	0	0
	MI	11.2	95.7	3.0	2.0	1.0	0	0	0	0	0	0
	MA	85	8.7	0.1	0.1	0.03	0	0	0	0	0	0
	NTP	281	287	3	4	1	0	0	0	0	0	0
	FD (%)	30.3	31.0	0.3	0.4	0.1	0	0	0	0	0	0
Gizzard	P (%)	9.1	9.1	0	0	0	0	0	0	0	0	0
	MI	10.7	0.3	0	0	0	0	0	0	0	0	0
	MA	1	0.03	0	0	0	0	0	0	0	0	0
	NTP	32	1	0	0	0	0	0	0	0	0	0
	FD (%)	3.5	0.1	0	0	0	0	0	0	0	0	0
Proventriculus	P (%)	12.1	0	6.1	0	0	0	0	0	0	0	0
	MI	17.3	0	3.5	0	0	0	0	0	0	0	0
	MA	2.1	0	0.2	0	0	0	0	0	0	0	0
	NTP	69	0	7	0	0	0	0	0	0	0	0
	FD (%)	7.5	0	0.8	0	0	0	0	0	0	0	0
Ventriculus	P (%)	0	0	12.1	0	0	0	0	0	0	0	0
	MI	0	0	2.3	0	0	0	0	0	0	0	0
	MA	0	0	0.3	0	0	0	0	0	0	0	0
	NTP	0	0	9	0	0	0	0	0	0	0	0
	FD (%)	0	0	1.0	0	0	0	0	0	0	0	0
Cecum	P (%)	0	0	0	0	0	0	0	54.5	3.3	0	0
	MI	0	0	0	0	0	0	0	10.5	2.0	0	0
	MA	0	0	0	0	0	0	0	5.7	0.2	0	0
	NTP	0	0	0	0	0	0	0	189	2	0	0
	FD (%)	0	0	0	0	0	0	0	20.4	0.2	0	0
Colon	P (%)	0	0	0	0	0	3.0	0	0	0	0	0
	MI	0	0	0	0	0	1.0	0	0	0	0	0
	MA	0	0	0	0	0	0.03	0	0	0	0	0
	NTP	0	0	0	0	0	1	0	0	0	0	0
	FD (%)	0	0	0	0	0	0.1	0	0	0	0	0
Intestine	P (%)	0	3.0	3.0	0	0	0	0	3.0	3.3	0	3.0
	MI	0	1.0	2.0	0	0	0	0	8.0	2.0	0	1.0
	MA	0	0.03	0.1	0	0	0	0	0.2	0.2	0	0.03
	NTP	0	1	2	0	0	0	0	8	2	0	1
	FD (%)	0	0.1	0.2	0	0	0	0	0.9	0.2	0	0.1

Table 2 continued

SI	Parameters	*Eucoleus contortus*	*Aniskatis* sp.	*Contracaecum* sp.	*Hysterolylacium* sp.	*Raphidascaris* sp.	*Eustrongylides* sp.	*Syngamus* sp.	*Capillaria* sp.	*Subulura* sp.	*Athesmia heterolecithodes*	*Ascocotyle* sp.
Trachea	P (%)	0	0	0	0	0	0	9.1	0	0	0	0
	MI	0	0	0	0	0	0	3.7	0	0	0	0
	MA	0	0	0	0	0	0	0.3	0	0	0	0
	NTP	0	0	0	0	0	0	11	0	0	0	0
	FD (%)	0	0	0	0	0	0	1.2	0	0	0	0
Gallbladder, hepatic ducts	P (%)	0	0	0	0	0	0	0	0	0	12.1	0
	MI	0	0	0	0	0	0	0	0	0	3.8	0
	MA	0	0	0	0	0	0	0	0	0	0.5	0
	NTP	0	0	0	0	0	0	0	0	0	1.5	0
	FD (%)	0	0	0	0	0	0	0	0	0	1.6	0

P Prevalence, *MI* Mean intensity, *MA* Mean abundance, *SD* Standard deviation, *TNP* Total number of parasites, *FD* Frequency of dominance

are not in direct competition, aggregated dispersion could allow the coexistence of parasites that would otherwise be excluded; and hence, more parasite species can coexist in a same host population (Salgado-Maldonado et al. 2019). In addition, Lester & McVinish (2016) reported that most parasites that are able to remain at one trophic level are less aggregated than those that pass through the food web.

Infection parameters (sensu Bush et al. 1997) traditionally used to quantify parasite populations or the severity of parasitic infections, are subject to variations (Poulin 2006), because such parameters can vary for a single avian species, influenced by factors such as the host diet and selective feeding on prey that are intermediate hosts in the environment, among other factors (Alexander & Mclaughlin 1997; Sanmartín et al. 2004; Junker et al. 2008; Santoro et al. 2012). Our results for *C. moschata domestica* indicated low infection levels by nematodes and trematodes, except for *E. contortus* and *Capillaria* sp., but no difference was found between males and females, because males and females had a similar diet and lifestyle. In addition, we found a higher overall prevalence of helminths (90.9%) than that reported by Mattos-Junior et al. 2008 for this same duck (56.5%) raised in Rio Janeiro State (Brazil), although these authors found that females presented a higher prevalence of endohelminths than males. We attribute this high prevalence and intensity of *E. contortus* and *Capillaria* sp. to the humid environment characteristic of the Amazon. This high prevalence and intensity of nematodes in *C. moschata domestica* can be related to the interactions of this avian with the soil, which is essential for the maintenance of the life cycle of many parasites such as *E. contortus*, where this avian ingests the intermediate hosts, possibly earthworms, besides the viable eggs in the environment (Carvalho et al. 2019).

We observe that in 36.3% of *C. moschata domestica* there was an occurrence of double parasite infection, generally with *E. contortus* or *Capillaria* sp., which may lead to body weight loss. This high prevalence of helminths can be a problem for duck raising by affecting their total protein content and, consequently, the full economic benefits of their production (Carvalho et al. 2019). Additionally, we found no correlation between endohelminth abundance and the body weight of *C. moschata domestica*, indicating that possibly the amount of foods consumed containing infective stages was not a factor responsible for such infection levels.

Free-range and extensively raised ducks are in constant contact with soil and aquatic habitats, which serve as an important reservoir and transmission for larval stages of parasite endohelminths species. Hence, terrestrial and aquatic invertebrates can become vectors for these parasites (Alexander & Mclaughlin 1997; Muhairwa et al. 2007; Junker et al. 2008; Carvalho et al. 2019). Wild fish

Table 3 Dispersion index (ID), statistic-d (*d*) and discrepancy index (D) for the parasite infracommunities in *Cairina moschata domestica* from the eastern Amazon (Brazil)

Species of parasites	ID	*d*	D	Type of dispersion
Anisakis sp.	1.52	1.81	0.54	Random
Eucoleus contortus	2.46	4.61	0.47	Aggregated
Capillaria sp.	2.70	5.20	0.62	Aggregated

Table 4 Body and diversity parameters for the endohelminth parasite in *Cairina moschata domestica* from the eastern Amazon (Brazil)

Parameters	Mean ± SD	Range
Weight (kg)	2.6 ± 0.9	1.2–4.5
Species richness of parasites	1.9 ± 1.3	0–6
Brillouin diversity index (*HB*)	0.4 ± 0.4	0–1.2
Evenness (*E*)	0.2 ± 0.2	0–0.5

species are infected by larvae of *Anisakis* sp., *Contracaecum* sp., *Hysterotylacium* sp., *Raphidascaris* sp., *Eustrongylides* sp., *Capillaria* sp. and *Ascocotyle* sp. because they are secondary intermediate or paratenic hosts for such helminths, while fish-eating avian are definite hosts (Moravec 1998; Barson & Marshall 2004; Knoff et al. 2013; Pinheiro et al. 2019).

Fish-eating birds are abundant in freshwater habitats in the eastern Amazon, as they are in most regions of the Amazon. However, we also found *Anisakis* sp., *Contracaecum* sp., *Hysterotylacium* sp., *Raphidascaris* sp., *Eustrongylides* sp., *Capillaria* sp., *E. contortus*, *Athesmia heterolecithodes* (Braun, 1899) Looss, 1899 and *Ascocotyle* sp. in *C. moschata domestica* raised in the Brazilian Amazon, where they are dead-end hosts (i.e., accidental hosts) for these endohelminths. Thus, it is possible that this duck also feeds on fish, which are intermediate hosts for these nematode species in the environment. *Athesmia heterolecithodes* is a ubiquitous, nearly cosmopolitan digenean species with a wide diversity of hosts that includes avian species from the Gruiformes, Charadriiformes, Cuculiformes, Falconiformes, Strigiformes, Ciconiiformes and Passeriformes from the Old World, Nearctic and Neotropical regions, as well as mammal species from the Marsupialia, Chiroptera, Carnivora and Rodentia (Digiani 2000). In general, infrapopulation of nematodes and digeneans in *C. moschata domestica* had few individuals, suggesting that the same diet variation that produces the low diversity in the endohelminths community limits both the probability and frequency of encounters with parasites at the individual level. Since this host does not specialize on any prey species, this fact further limits exposure to helminth larvae. This is reflected in the generally low prevalence and intensity seen for most helminth species.

In *C. rothschildii*, *P. gularis* and *T. episcopus* of captivity in the city of Belém, State of Pará (Brazil) was reported eggs of nematode Trichostrongyloidea (4.6%), Ascaridoidea (0.6%) and Trichuroidea (0.6%); eggs of cestodes in 2.9% of examined birds and trematode eggs in 2.3% of samples. As species of *Anisakis*, *Contracaecum*, *Hysterotylacium*, *Raphidascaris*, *Eustrongylides* and *Capillaria* are widely distributed in other parts of world (Gower 1939; Alexander & Mclaughlin 1997; Moravec 1998; Barson & Marshall 2004; Sanmartín et al. 2004; Navarro et al. 2005; Muhairwa et al. 2007; Santoro et al. 2012), it is also important to investigate the occurrence of these helminths in the Amazon region. In addition, *Anisakis* sp., *Contracaecum* sp., *Eustrongylides* sp. and *Ascocotyle* sp. have zoonotic potential for humans (Knoff et al. 2013) due to the risk of contamination by larvae in the food of Amazon populations. This means that studies of infections by such parasites should not be neglected.

Conclusions

This study was characterized by a high richness of nematode species and a small number of digeneans with low abundance, low diversity, and low evenness, with a predominance of *E. contortus* and *Capillaria* sp. There was a predominance of single or double parasitic infection, generally with *E. contortus* and/or *Capillaria* sp. Our studies of these ducks raised in the Brazilian Amazon region revealed that they are accidental hosts for most of the larvae species, and definitive hosts for few of the species found. Furthermore, results suggest that *C. moschata domestica* is an omnivorous species that uses several aquatic and terrestrial habitats that contain a rich nematode fauna related to these two habitats, but mainly prefers aquatic habitats where there is greater opportunity for contact and consumption of invertebrates and fish infected with infectious stages of parasites. This was the first report of *Contracaecum* sp., *Hysterotylacium* sp., *Raphidascaris* sp., *Eustrongylides* sp., *Syngamus* sp., *Ascocotyle* sp. and *A. heterolecithodes* for *C. moschata domestica*. Because

this duck is an important source of food for several human populations in northern Brazil, knowledge regarding contamination by helminths found to have zoonotic potential is particularly important.

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Declaration

Conflict of interest Authors declare that there is no conflict of interest regarding the publication of this paper.

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ARTIGO 3

Título: A new nematode of the family Capillariidae identified in *Cairina moschata* (Linnaeus) on Marajó Island in the Brazilian Amazon

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Original Article

A new nematode of the family Capillariidae identified in *Cairina moschata* (Linnaeus) on Marajó Island in the Brazilian Amazon

Um novo nematoda da família Capillariidae identificado em *Cairina moschata* (Linnaeus) na Ilha de Marajó na Amazônia Brasileira

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Abstract

Capillaria Zeder, 1800, parasitizes the organs and tissues of several hosts, including the domestic duck *Cairina moschata* (Linnaeus). This article describes a new species of *Capillaria* in domestic ducks identified based on morphological studies and molecular analyses of the ribosomal RNA gene. Thirty-eight specimens of *C. moschata* from the municipality of Soure, Marajó Island, Pará, Brazil. The organs of the birds' digestive tract were analyzed under a stereomicroscope to confirm the parasitic infection, after which the collected nematodes were identified by light microscopy, scanning electron microscopy, and molecular analysis. Capillariids parasitized the intestine and cecum of the examined birds. These parasites had three bacillary bands and a pair of elongated precloacal papillae on the tail. Phylogenetic analysis indicated that the new species formed a sister clade with *Capillaria spinulosa* (Linstow, 1890), as described in Indonesia and Japan. Based on morphological distinctions and molecular data, *Capillaria cairina* n. sp. can be considered a new parasite species of *C. moschata* in the Brazilian Amazon.

Keywords: *Capillaria cairina* n. sp., Anseriformes, Muscovy duck, Brazil.

Resumo

Capillaria Zeder, 1800 parasitam órgãos e tecidos de diversos hospedeiros, incluindo o pato doméstico *Cairina moschata* (Linnaeus). Este artigo descreve uma nova espécie de *Capillaria* em patos domésticos, identificada com base em estudos morfológicos e análises moleculares do gene do RNA ribossomal. Trinta e oito espécimes de *C. moschata* oriundo dos no município de Soure, Ilha de Marajó, Pará, Brasil. Os órgãos do aparelho digestivo das aves foram analisados em estereomicroscópio para confirmar a infecção parasitária. Após, os nematódeos coletados foram identificados por microscopia de luz, microscopia eletrônica de varredura e análises moleculares. Os capilarídeos parasitaram intestino e ceco das aves examinadas. Esses parasitas apresentaram três bandas bacilares e um par de papilas pré-cloacais alongadas na cauda. A análise filogenética indicou que a nova espécie formou um clado irmão com *Capillaria spinulosa* (Linstow, 1890), conforme descrito na Indonésia e no Japão. Com base na distinção morfológica e dados moleculares, *Capillaria cairina* n. sp. pode ser considerada uma nova espécie de parasita de *C. moschata* na Amazonia brasileira.

Palavras-chave: *Capillaria cairina* n. sp., Anseriformes, Pato doméstico, Brasil.

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Introduction

Understanding the diversity and biology of nematodes of the family Capillariidae Railliet, 1915 remains scarce. Approximately 390 species have been identified in various organs and tissues in many hosts, such as fish, amphibians, reptiles, birds, and mammals, including humans (Vicente et al., 1995; Moravec, 2001a; Hodda, 2011). However, capillariids are often not identified in faunal surveys and have been reported only as *Capillaria* (*sensu latu*) sp. or Capillariidae gen. sp. (Moravec & Beveridge, 2017; Moravec & Justine, 2020).

The taxonomic classification and identification of capillariid nematodes are difficult as they are small, fragile parasites that require intense manipulation for observation (Moravec, 1982; Anderson, 2000; Tamaru et al., 2015). In addition, they exhibit a wide range of ecological adaptation mechanisms, parasitizing vertebrates belonging to the main classes in both terrestrial and aquatic conditions (fresh and marine water). Although many forms have adapted to life in the tissues of various organs (Moravec, 2001b), most capillariids are digestive tract parasites. Deng et al. (2021) demonstrated that using molecular markers to study phylogenetic and systematic relationships within the family Capillariidae is helpful in investigating controversial taxonomic problems in the identification of genera or species.

Muscovy ducks, *Cairina moschata* (Linnaeus), are well adapted to different climatic conditions and have adjusted to breeding in captivity (Béjcek & Stastný, 2002). However, they are also found in the wild, in areas with adequate water and space (Geromel, 2012). Subsistence farming is common in Brazil, and the trade of live birds, eggs, and meat occurs mainly among small producers, commercial houses, and open markets (Almeida et al., 2016). On Marajó Island, birds are raised extensively, making these animals generalists in their diet; thus, they can act as paratenic hosts by ingesting infected fish viscera or becoming infected after filtering water containing microcrustaceans parasitized by nematodes or eggs (Carvalho et al., 2020). On Marajó Island, Muscovy ducks are commonly used by the local population for food and commercial purposes (Carvalho et al., 2019, 2021).

In Brazil, four capillariid species have been identified among the parasitic fauna of *C. moschata* as follows: *Capillaria phasianina* Kotlán, 1914; *Capillaria* sp. Pinto and Almeida, 1935, and *Eucoleus cairinae* (Freitas & Almeida, 1935) Lopez & Neyra, 1947, in Rio de Janeiro; and *Eucoleus contortus* Creplin, 1989, in Pará (Vicente et al., 1995; Mattos et al., 2008; Carvalho et al., 2019). This study describes a new nematode species of the genus *Capillaria* Zeder, 1800, that was found to infect Muscovy ducks in the Brazilian Amazon based on morphological data and phylogenetic analyses.

Material and Methods

Sample collection

From 2018 to 2020, 38 *C. moschata* (25 females and 13 males) specimens were acquired from rural properties located in the municipality of Soure (00° 43' 00" S; 48° 31' 24" W), Marajó Island, Pará State. Only digestive tract organs were transported to the laboratory at the Universidade Federal Rural da Amazônia, Campus Belém, Pará.

Parasitological examination

Each organ was isolated in a petri dish containing 0.9% NaCl saline in the laboratory and analyzed under a stereomicroscope (Leica ES2). Collected nematodes were washed in 0.9% saline, fixed in an A.F.A. solution (93 parts 70% ethyl alcohol, five parts formaldehyde, and two parts glacial acetic acid), and stored in 70% alcohol. In total, 197 adult nematodes were collected (114 females and 83 males), of which 118 were from the ceca, and 79 were from the large intestine. For light microscopy, nematodes were cleared in 0.5% lactophenol amine solution, observed under a Leica DM2500 microscope with a drawing tube, imaged under a Leica DM2500 microscope with a Leica DFC310 FX camera system using Leica Application Suite Software V4.4, and stored in glycerin alcohol (50% of 70% ethyl alcohol and 50% glycerin). For the morphometric analysis, 20 males, 20 females, and 50 eggs were used. Measurements are given in micrometers unless otherwise indicated and are represented as mean values, followed by the minimum and maximum values in parentheses.

Scanning electron microscopy (SEM)

Forty-five nematodes (22 females and 23 males) were fixed in 3% glutaraldehyde, washed in 0.2 M phosphate buffer solution, post-fixed in 1% osmium tetroxide for 2 h, dehydrated in progressive alcohol for 1 h each (50%, 70%, 80%, 90%, and 100%), dried at the critical point of CO_2, metalized with gold-palladium, and observed using a Vega 3 (TESCAN, Brno, Czech Republic) scanning electron microscope, according to Carvalho et al. (2022).

Molecular analysis

For molecular and phylogenetic analyses, 20 nematodes (10 females and 10 males) were used. Helminths were isolated from the ceca and fixed in absolute alcohol. Total DNA was extracted using the Purelink® Genomic DNA Mini Kit (Invitrogen®; ThermoFisher, CA, USA), following the manufacturer's instructions. The small subunit ribosomal RNA gene (SSU rDNA) sequence was amplified using primers 18S-E/18S-A27 and 18S-8/Cestode-6 (Olson & Caira, 1999). The final volume for the polymerase chain reaction (PCR) was 25 µL, containing 1 ng of DNA template, 20 mM Tris pH 8.4, 50 mM KCl, 2 mM dNTP (Invitrogen®), 1 mM Mg_2Cl, 0.5 pmol of each primer, and 0.2 U of Taq DNA polymerase (Invitrogen®). The amplification profile for the polymerization of any molecules that might have dissociated from the polymerase prior to complete fragment synthesis consisted of 5 min of initial denaturation at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C, with a final extension of 7 min at 72 °C.

The amplicons were subjected to 1.5% agarose gel electrophoresis and purification using ExoSAP-ITTM (GE Healthcare, UK). Quantification was performed using Nanodrop (ThermoFisher, CA, USA). The samples were sequenced using an Applied Biosystems™ 3500 Genetic Analyzer (ThermoFisher, CA, USA), which generated approximately 700 nucleotides in each sequence. The primers used for PCR amplification and sequencing were as follows: 18S-E forward (5'-CCG AAT TCG ACA ACC TGG TTG ATC CTG CCA GT-3')/18S-A27 reverse (3'-CCA TAC AAA CGT CCC CGC CTG -5') and 18S-8 forward (5'-GCA GCC GCG GTA ATT CCA GC-3')/18S-Cestode 6 reverse (3'-ACG GAA ACC TTG TTA CGA CT-5').

The nucleotide sequences obtained from the samples were edited and aligned using BioEdit software (Hall et al., 2011) after a comparison with other sequences available in GenBank (BLAST search). The SSU rDNA sequence was aligned with sequences of 21 capillariids available in GenBank. In addition, the database includes sequences from *Haemonchus placei* (Place, 1893) and *Haemonchus contortus* (Rudolphi, 1803), which formed the outgroup for phylogenetic analyses. The consensus nucleotide sequences reported in this study are available in GenBank under accession number OP720889.

Bayesian inference (BI) was performed using a Markov Chain Monte Carlo (MCMC) phylogenetic tree, implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). This analysis was based on two parallel runs of four simultaneous MCMC searches of five million generations each. One tree was sampled every 250 generations after discarding the first 1000 trees as burn-in. The remaining trees were analyzed using MrBayes to estimate the posterior probability of each node in the phylogenetic reconstruction. As indicated by jModelTest 2.1.9 (Darriba et al., 2012), the BI analysis assumed a TIM3ef + I + G nucleotide substitution model, with estimated base frequencies (A = 0.2571, C = 0.2091, G = 0.2751, and T = 0.2587), the replacement model (A–C = 0.6282, A–G = 2.3543, A–T = 1.0599, C–G = 0.6321, C–T = 3.8687, G–T = 1.0000), and local variables after a gamma distribution (G = 0.3820), and 88 models in the 100% confidence interval. In addition, genetic distances were determined for the SSU rDNA sequences of the capillariid species using PAUP 4.0 (Swofford, 1998). Specimens of capillariids were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Manguinhos, Rio de Janeiro, Brazil, as follows: holotype (CHIOC 39393 a), allotype (CHIOC 39393 b), and paratypesfour males (CHIOC 39393 c-f) and four females (CHIOC 39393 g-j).

Results

Description

Trichinellida Hall, 1916

Capillariidae Railliet, 1915

Capillaria Zeder, 1800

Capillaria cairina n. sp.

(Based on light microscopy and SEM, Figures 1-4)

The nematodes were small and filiform, with delicate cuticles that were transversely striated. They exhibited a simple, dorsoventrally oriented oral opening. Their mouth was surrounded by 12 cephalic papillae arranged in two circles, each consisting of six papillae and a pair of small lateral amphids, with a stylet absent. They contained a short, muscular esophagus with a stichosome. The nerve ring surrounded the muscular esophagus in its initial portion. The stichosome consisted of a single row of approximately 40 elongated stichocytes with transverse rings that were difficult to visualize and large stichocytes nuclei with several nucleoli.

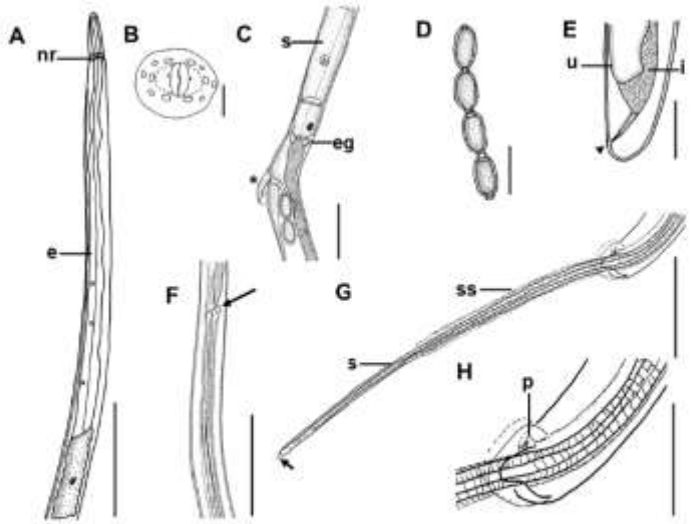

Figure 1. *Capillaria cairina* n. sp. (A) Anterior extremity, muscular esophagus (e), and nerve ring (nr). Scale bar = 10 µm; (B) Detail of the male's anterior extremity, prominent lips surrounded by 12 papillae, apical view (reconstructed from an SEM micrograph). Scale bar = 2 µm; (C) End of stichocytes (s), esophageal glands (eg), and pre-equatorial region where the vulva and vulvar appendix are located (*). Scale bar = 10 µm; (D) Eggs. Scale bar = 40 µm; (E) Female posterior extremity, lateral view, anal opening (arrowhead); the uterus (u) and intestine (i) are shown. Scale bar = 50 µm; (F) Region where the base of the spicule is observed (arrow). Scale bar = 10 µm; (G) Posterior extremity of male, lateral view, spicule (s), spinous spicular sheath (ss), and rounded tip of the spicule (arrow). Scale bar = 10 µm. H. Posterior extremity of male showing papilla (p). Scale bar = 5 µm.

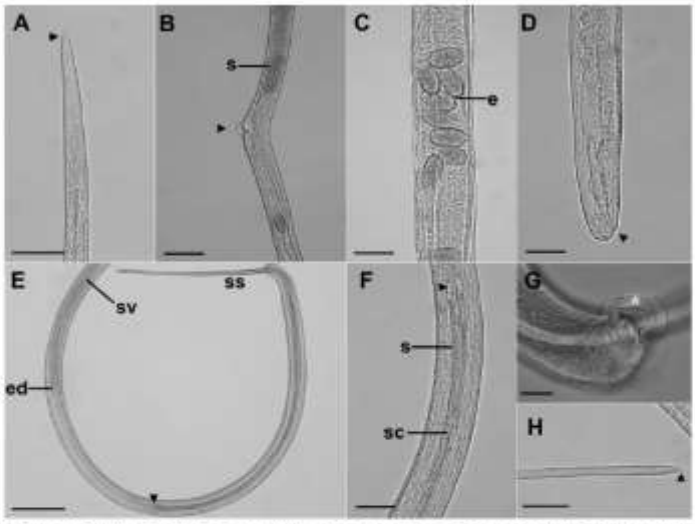

Figure 2. Microscopic views of *Capillaria cairina* n. sp. (A) Female anterior end (arrowhead). Scale bar = 50 µm; (B) Pre-equatorial region, lateral view, where the stichocytes (s) end and the vulva with its vulvar appendix (arrowhead). Scale bar = 100 µm; (C) Uterus with eggs (e) with clearly visible polar plugs. Scale bar = 50 µm; (D) Posterior extremity, lateral view; the anal opening is observed (arrowhead). Scale bar = 50 µm; (E) Posterior extremity of the male, lateral view; the base of the spicule (arrowhead) and extruded spicular sheath (ss) can be observed. Scale bar = 200 µm; (F) Base of the spicule (arrowhead). Scale bar = 50 µm; (G) Posterior extremity of the male, lateral view, lobe with a delicate membrane (*). Scale bar = 20 µm; (H) Distal end of the spicule with a rounded tip and the presence of a thin membrane at its apex (arrowhead). Scale bar = 50 µm. s = spicule; ss = spicular sheath; sc = spicular canal; ed = ejaculatory duct; sv = seminal vesicle.

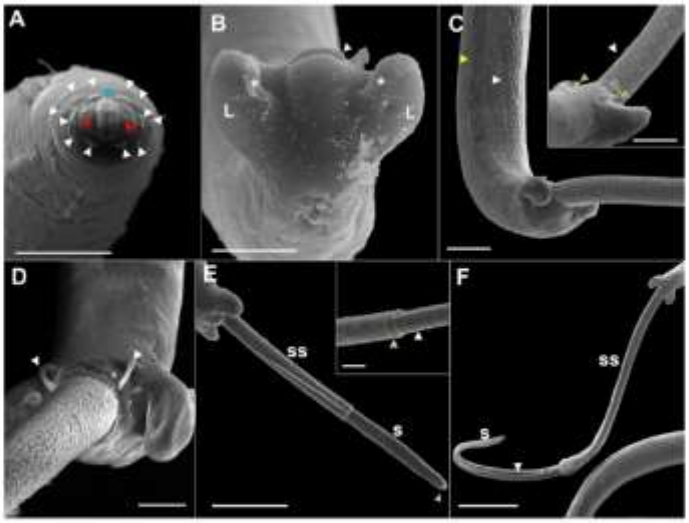

Figure 3. Scanning electron microscopy of male *Capillaria cairina* n. sp. (A) Detail of the male's anterior extremity, prominent lips (blue arrowhead) surrounded by 12 papillae (white arrow), apical view, and pair of small lateral amphids (red arrowhead). Scale bar = 5 µm; (B) Posterior extremity, ventral view, cloacal opening (arrowhead), membrane (*) and the caudal lobes (L). Scale bar = 20 µm; (C) Posterior extremity, lateral and ventral views of the tail with an extruded spicular sheath; lateral (yellow arrowhead) and ventral (white arrowhead) bacillary bands can be observed. Scale bar = 20 µm. In the insert, note the digitiform pre-cloacal papillae (green arrowhead) and the base of the spineless spicular sheath (white arrowhead). Scale bar = 20 µm; (D) Note the digitiform pre-cloacal papillae (arrowhead). Scale bar = 10 µm; (E) Posterior end with partially extruded spinous spicular sheath (ss), rough spicule (s), and membrane of the distal end of the spicule (blue arrow). Scale bar = 50 µm. Insert showing the distal end of the spicular sheath with spines (green arrowhead) and rough spicule (arrowhead). Scale bar = 10 µm; (F) Posterior extremity with spicule (s) and spicular sheath (ss) with fully extruded spines; the spicular canal is observed (arrowhead). Scale bar = 100 µm.

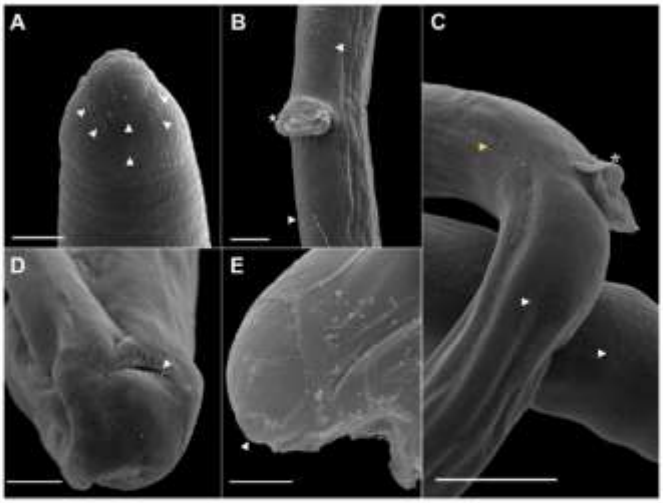

Figure 4. Scanning electron microscopy of female *Capillaria cairina* n. sp. (A) Anterior end, large papillae (white arrowhead). Scale bar =2 µm; (B) The vulva is observed in the pre-equatorial region, with the presence of a vulvar appendix (*) and a rectangular area of interruption of the ventral bacillary bands (arrowhead). Scale bar = 20 µm; (C) Lateral (yellow arrowhead) and ventral (white arrowhead) views of the bacillary band and observed vulvar appendix (*). Scale bar = 50 µm; (D) Posterior extremity, lateral and ventral views of the tail; the anal opening is observed (arrowhead). Scale bar = 10 µm; (E) Observation of the egg with a polar plug (arrowhead) still in the vulvar appendix. Scale bar = 10 µm.

Two glandular cells were observed at the esophageal–intestinal junction. Lateral and ventral bacillary bands extended for almost the entire body length in both sexes (Figures 1A-B, G).

Male measurements (based on 20 specimens with an extruded spicular sheath, holotype measurements in brackets) were as follows: body length, 14.7 (11.8–15.8) mm [15.3 mm]; maximum width at the esophageal–intestinal junction, 43 (36–51) [45]; muscular esophagus measuring 335 (256–366) [338] × 14 (13–15) [15] wide; bacillary bands lateral and ventral to the body; total esophagus length, 5.7 (5.0–6.2) mm [6.2 mm], representing 39 (35–41) % [41%] of the body length; stichosome length, 5.4 (4.7–5.9) mm [5.9 mm]; the number of stichocytes, 41 (37–41) [39]; 13 (7–17) [7] transverse rings. The nerve ring was situated 69 (41–70) [70] from the anterior extremity. They exhibited a single, heavily sclerotized spicule measuring 1.7 (1.5–1.7) mm [1.6 mm] × 22 (16–23) [23] wide, representing 12 (10–11) % [10%] of the body length, with a spinous spicular sheath measuring 280 (123–433) [286] × 18 (13–16) [16]. The sheath was not spiny at the base. A well-developed spicular canal was observed. The proximal end of the spicule was expanded, and the distal end was rounded, with rough transverse grooves observed on the spicule surface. The caudal end was rounded and bifurcated in ventral and dorsal views, without a pseudobursa, supporting two large, round ventrolateral lobes containing a pair of sessile ventrolateral pre-cloacal papillae and a terminal cloacal opening (Figures 1F-G, 2E-H, 3A-F).

Female measurements (based on 20 gravid specimens, allotype measurements in brackets) were as follows: body length, 23.1 (16.7–28.6) mm [24.6 mm]; maximum width at the esophageal–intestinal junction, 45 (38–53) [45]; bacillary bands lateral and ventral to the body; total length of the esophagus, 6.7 (5.4–8.0) mm [7.9 mm], representing 29 (26–33) % [32%] of the body length; length of the muscular esophagus, 380 (335–445) [401] × 15 (13–20) [13] wide; stichosome length, 6.3 (5.1–7.6) mm [7.4 mm]; the number of stichocytes, 40 (37–42) [40], with 14 (11–23) [12] transverse rings. The nerve ring was situated 72 (46–95) [76] from the anterior extremity. The pre-equatorial vulva was located 7.7 (5.5–12.0) mm [7.9 mm] from the anterior extremity, with vulvar lips exhibiting appendages. The vagina was short and muscular. Eggs were barrel-shaped and arranged in a single row near the vulvar passageway, measuring 42 (35–48) [42] × 20 (18–22) [20] with projecting polar plugs. Females exhibited rounded caudal ends with a subterminal anus (Figures 1B-D, 2B-D, 4A-E).

Molecular and phylogenetic analyses

The partial rDNA sequence obtained for *Capillaria cairina* n. sp. was 1750 bp in length and is available in GenBank (OP720889). Thirty-one taxa were used for the comparison, and the outgroups were *H. contortus* and *H. placei*. Among these, four large, well-supported clades were formed as follows: A (*Capillaria*), B (*Eucoleus* Dujardin, 1845), C (predominantly *Aonchotheca* López-Neyra, 1947), and D (*Baruscapillaria* Moravec, 1982). A BLAST search revealed that the nucleotide sequences with the highest similarity were those of *Capillaria pudendotecta* Lubimova, 1947 (accession numbers LC052338 and LC052339), observed in swans in Japan, with 95.45% and 95.53% similarity, respectively, and of *Capillaria spinulosa* (Linstow, 1890) (accession numbers LC424999 and LC425000), in domestic birds from Indonesia and Japan, with 95.32% similarity. Clade A formed two subclades, A1 and A2, in which parasites belonging to the genus *Capillaria* were observed in the following hosts: Anseriformes (Anatidae), Galliformes (Phasianidae), Passeriformes (Corvidae), and Accipitriformes (Accipitridae), with infection sites in the ceca and small intestine (Figure 5).

Remarks: The analysis of clade A and subclades A1 and A2 was performed, as the parasites were of the same genus as those in our study and differed due to the morphological characteristics observed in Table 1, with SEM and molecular biology being fundamental for the identification of *Capillaria cairina* n. sp.

Capillaria cairina n. sp., observed in subclade A1 in this study, formed a sister clade with *C. spinulosa* with a genetic distance of 6%, which can be justified as these parasites infect hosts of the same Anseriformes order and Anatidae family. *Capillaria cairina* n. sp. has morphological characteristics that distinguish it from *C. spinulosa* (Table 1), and it is the only family in this clade to occur in the ceca and large intestine of Muscovy ducks in Brazil. In this subclade, *C. pudendotecta* had a genetic distance of 6.4% to the results of our study. However, the only similarity was observed in Anseriformes and Anatidae birds.

Subclade A2 contained *Capillaria anatis* (Schrank, 1790), *Capillaria madseni* Wakelin, Schmidt & Kuntz, 1970, and *Capillaria tenuissima* (Rudolphi, 1803) (with genetic distances of 16%, 17.1%, and 21,8%, respectively), which are parasites of Galliformes observed in the ceca and small intestine. In contrast, Accipitriformes and Passeriformes birds from Japan and Germany were infected only in the small intestine, thus differing from in our study.

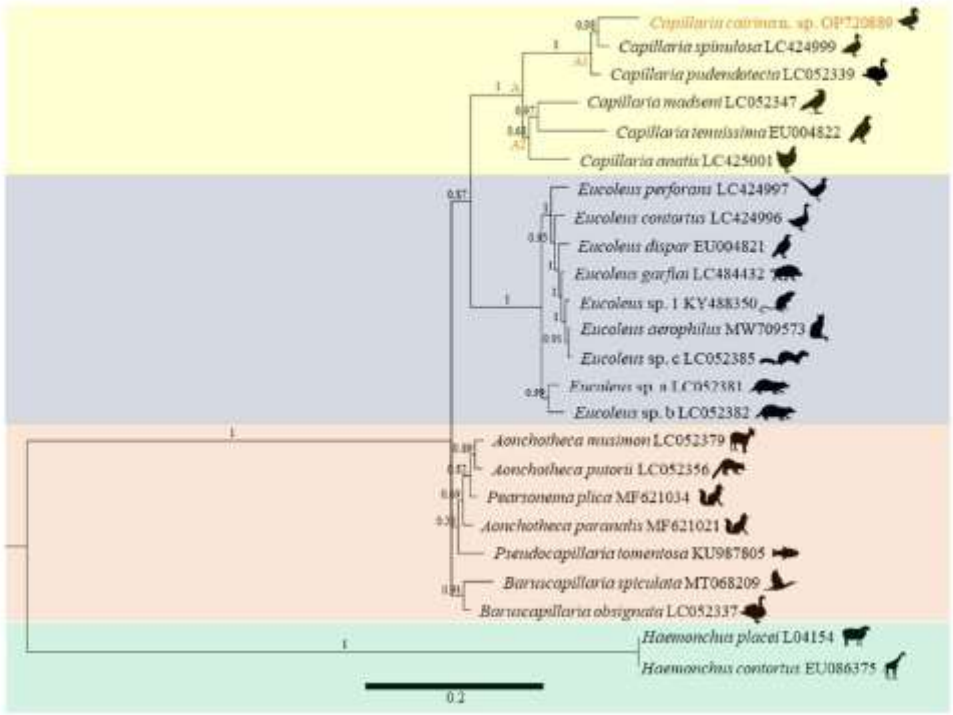

Figure 5. Bayesian phylogenetic tree based on SSU rDNA sequence obtained from *Capillaria cairina* n. sp., compared to that of other capillariids. Node numbers represent probability values calculated from BI/bootstrap ML values (> 50%). The scale bar indicates the number of mutations per site. Data are displayed with the species names, followed by the images of the hosts.

Taxonomic summary

Host type: *Cairina moschata* (Anseriformes: Anatidae).

Host site: large intestine and ceca.

Location: Municipality of Soure (00° 43' 00" S; 48° 31' 24" W), State of Pará, Brazilian Amazon.

Parasite data: prevalence, 47.4%; total worm recovery, 197; intensity, 2–38 (10.9 on average); abundance, 5.18.

ZooBank registration: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Capillaria cairina* n. sp. is urn: lsid: zoobank.org:pub: XXXXX

Etymology: The specific name *cairina* (genitive) is related to the generic name of the host bird (*Cairina*).

Discussion

Baruš et al. (1978) recorded three species of *Capillaria* parasitizing piscivorous birds from Europe and Asia based on the site of infection and length of the male's spicular sheath. Thus, the species *C. anatis*, *Capillaria spirale* (Molin, 1858) Travassos, 1915, and *Capillaria contorta* (Creplin, 1839) were identified parasitizing birds of the orders Podicipediformes, *Podiceps grisegena* (Boddaert); Anseriformes, *Mergus merganser* (Linnaeus) and *Mergusserrator* (Linnaeus); and Charadriiformes, *Larus argentatus* (Pontoppidan), *Larusminutus* (Pallas), *Larusridibundus* (Linnaeus), *Hydroprogne caspia* (Pallas), *Thalasseus sandvicensis* (Latham), and *Sternula albifrons* (Pallas). Despite the wide distribution of these parasites in birds, few studies still identify these capillariids at the specific level (Dar et al., 2013; Stapf et al., 2013; Carvalho et al., 2019).

Table 1. Data on the distance *p* calculated for the capillariid species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Present study	-																					
Capillaria madseni	0.171	-																				
Capillaria pudendotecta	0.064	0.153	-																			
Capillaria anatis	0.163	0.099	0.135	-																		
Capillaria tenuissima	0.218	0.136	0.194	0.153	-																	
Capillaria spinulosa	0.060	0.150	0.029	0.151	0.201	-																
Eucoleus sp. a	0.264	0.212	0.231	0.195	0.251	0.243	-															
Eucoleus sp. b	0.271	0.215	0.246	0.207	0.247	0.25	0.028	-														
Eucoleus sp. 1	0.207	0.145	0.165	0.144	0.217	0.191	0.027	0.030	-													
Eucoleus sp. c	0.285	0.232	0.250	0.215	0.274	0.261	0.039	0.043	0.004	-												
Eucoleus contortus	0.263	0.210	0.232	0.197	0.248	0.241	0.039	0.041	0.012	0.021	-											
Eucoleus dispar	0.270	0.232	0.261	0.217	0.270	0.271	0.043	0.053	0.008	0.020	0.023	-										
Eucoleus aerophilus	0.288	0.230	0.253	0.213	0.271	0.262	0.040	0.045	0.003	0.003	0.020	0.021	-									
Eucoleus gorfai	0.275	0.206	0.241	0.192	0.248	0.252	0.035	0.040	0.004	0.010	0.017	0.015	0.008	-								
Eucoleus perforans	0.276	0.222	0.247	0.214	0.268	0.259	0.046	0.048	0.021	0.029	0.030	0.034	0.031	0.027	-							
Aonchotheca paranalis	0.236	0.156	0.193	0.158	0.214	0.203	0.142	0.135	0.120	0.161	0.143	0.158	0.159	0.148	0.160	-						
Aonchotheca musimon	0.239	0.161	0.188	0.153	0.213	0.203	0.129	0.130	0.109	0.148	0.136	0.147	0.148	0.146	0.139	0.033	-					
Aonchotheca putorii	0.237	0.153	0.191	0.148	0.211	0.205	0.127	0.129	0.113	0.152	0.130	0.149	0.151	0.146	0.139	0.031	0.019	-				
Pearsonema plica	0.233	0.154	0.194	0.151	0.202	0.201	0.136	0.128	0.113	0.152	0.137	0.145	0.152	0.141	0.146	0.024	0.021	0.022	-			
Pseudocapillaria tomentosa	0.222	0.147	0.190	0.165	0.223	0.199	0.152	0.145	0.126	0.163	0.151	0.160	0.164	0.145	0.165	0.045	0.051	0.051	0.046	-		
Baruscapillaria spiculata	0.251	0.152	0.205	0.159	0.217	0.217	0.164	0.164	0.118	0.186	0.166	0.170	0.184	0.160	0.177	0.070	0.073	0.074	0.067	0.074	-	
Baruscapillaria obsignata	0.205	0.123	0.157	0.121	0.181	0.170	0.114	0.114	0.102	0.128	0.117	0.131	0.128	0.140	0.125	0.039	0.039	0.038	0.038	0.044	0.036	-

According to Moravec (1982) and Vicente et al. (1995), the genus *Capillaria* has the following diagnostic characteristics: the caudal lateral wings are absent in males; the posterior end of the male is rounded and equipped with two lateral lobes, located ventrolaterally or dorsolaterally. This genus lacks a membranous bursa, and sessile pre-cloacal papillae are often observed. These nematodes exhibit a sclerotized spicule and spiny spicular sheath, and they may or may not exhibit a vulvar appendix. They are intestinal parasites of fish, amphibians, reptiles, birds, and mammals, with thetype species being *C. anatis*. The parasite observed in *C. moschata* in this study exhibited characteristics of *Capillaria*. Baruš et al. (1981) identified *C. anatis* in *Anas acuta* (Linnaeus) and observed that the posterior end of the male forms a pseudobursa with a smooth cuticle and that the base is formed by two well-developed and rounded lateral processes, with a rounded cloacal opening located ventrally. The species identified in our study had a pair of sessile, longitudinally elongated pre-cloacal papillae, transverse cloacal openings, and lobes without pseudobursa; however, each had a well-marked membrane (Figure 2G).

By light microscopy, the parasite seemed to be similar to *C. anatis*, *C. spinulosa*, and *C. pudendotecta*. However, with SEM, our capillariid showed three bacillary bands (lateral and ventral), a delicate membrane in each lobe, and a pair of elongated pre-cloacal papillae, which differed from the descriptions of *C. anatis* reported by Baruš et al. (1978, 1981). They observed microscopically the posterior end of males, and they could observe a delicate membrane between ventro-lateral lobes, called "pseudobursa," and a pair of short pre-cloacal papillae in males. The description differed from that of *C. spinulosa*, provided by Mettrick (1959) and Sakaguchi et al. (2020), who observed males with a caudal end showing two small, non-elongated papillae. Oyarzún-Ruiz et al. (2019) identified the presence of a spiny sheath with a distal expansion without a thorn in males of *C. pudendotecta*, which differed from the description of males in our study (Figure 4F) since the absence of spines was at the base of the sheath.

All female specimens in our study were morphologically similar to *C. pudendotecta* described by Oyarzún-Ruiz et al. (2019), with a small vulvar appendix. However, they could be easily distinguished from *C. anatis* and *C. spinulosa* described by Baruš et al. (1981), Tanveer et al. (2015), and Yevstafieva et al. (2020). They identified females without a vulvar appendix, and Mettrick (1959) and Sakaguchi et al. (2020) reported that *C. spinulosa* females had a vulva without a vulvar appendix. These differed from the females in our study but corroborated Moravec's (2001b) observations, who reported that capillariid females may or may not have a vulvar appendix. Thus, the organisms identified in this study differed from those already registered (Table 2).

Tamaru et al. (2015) and Sakaguchi et al. (2020) recorded *C. pudendotecta* and *C. spinulosa*, respectively, only in the ceca of *Anas platyrhynchos* var. *domesticus* (Linnaeus), *Anser cygnoides domesticus* (Linnaeus), and *Cygnus olor* (Gmelin). The morphometric comparisons with other capillariids that parasitize the large intestine and ceca of birds are described in Table 2. Yevstafieva et al. (2020) reported specific morphological characteristics and biometric parameters of male and female *C. anatis* observed in *A. platyrhynchos* raised on poultry farms in Ukraine. As reported by Moravec (1982, 2001b) and Melnychuk et al. (2020), males have characteristics such as a pseudobursa, spicules, and spicular sheath ornamentation, and in females, the morphology of the vulvar area and eggs in the uterus should be considered.

Cram (1936) and Moravec (2001b) considered the shape and arrangement of hypodermic cells (bacillary bands) as a complementary characteristic for the differentiation between genera and species of capillaries. In the capillariids identified in this study, three bacillary bands were observed in both males and females, which differed from those observed in other species. In addition, the male was reported to have a spicular sheath with sclerotized spines oriented toward the cloaca. The spines were sparse in the proximal part of the spicule sheath, whereas they were more densely distributed in the middle and distal parts.

The morphological details of capillariids, such as the anterior extremity, bacillary bands, and tail, are not visible under light microscopy (Moravec, 2001a; Moravec & Barton, 2018; Carvalho et al., 2019). In this study, we observed the conical shape of the cephalic region with large and flat papillae in both males and females using SEM. Females have a well-defined vulvar appendix and a pair of papillae close to the vulva. In addition, a large "rectangular" area without a bacillary band was observed around the vulva. The tail of the male exhibited a pair of thin and elongated papillae, a spicular sheath armed with small spines, and three bacillary bands, with the ventral bacillary band being wider in females. None of these aspects have been previously reported in capillariid species and are described for the first time in the genus *Capillaria* in our study (Figures 3C–D).

The descriptions of many capillariids and the establishment of unjustified new genera within the family Capillariidae have confused the taxonomy and systematics of this group of parasites (Moravec et al., 1981).

Table 2. Comparison of the morphometric characteristics of *Capillaria cairina* n. sp. with other species of the genus *Capillaria* that present spicular sheath in Brazil found in the intestines and ceca of birds.

Morphometric characterization	*Capillaria cairina* n. sp.		*C. anatis*		*C. anatis*		*C. longicollis*		*C. nyrocinarum*		*C. spinulosa*		*C. tenuissima*	
Specimen sex	**Male**	**Female**	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	***Cairina moschata***		*Anas querquedula, Anas crecca, Anas platyrhynchos, Fulica atra*		*Anser anser anser*		Pigeon, little bustard and several gallinaceous birds		Eider – ducks		Wigeon and common scoter		Owls	
Locality	**Soure, Pará, Brazil**		Zoological Museum of Copenhagen, Denmark		Hertfordshire		British Museum		Escotland		British Museum		Britain	
Total body (L) [a]	**11.8–15.8**	**16.7–28.6**	7.0–12.2	8.4–14.9	6.2–10.7	7.1–12.9	8.4–14.7	8.9–17.6	9.7–11.6	7.6–13.4	7.8–8.5	9.8–13.1	8.7–11.3	18.2–23.3
Maximum body (W) [b]	**36–51**	**38–53**	–	–	54	66	68	73	78	88	48	62	59	69
Nerve-ring (L) [a,c]	**41–70**	**46–95**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (L) [a]	**256–366**	**335–445**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (W) [a]	**13–15**	**13–20**	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [a,c]	**5.0–6.2**	**5.4–8.0**	–	–	–	–	–	–	–	–	–	–	–	–
Vulva (L) [a]	–	**5.5–12.0**[e]	–	2.1–2.7[e]	–	0.036–0.042[e]	–	0.030–0.070[e]	–	–	–	0.066–0.072[e]	–	–
Vulvar appendage	–	**Present**	–	–	–	Absent	–	Present	–	Present	–	Absent	–	Absent
Egg (L × W) [a]	**35–48 × 18–22**		49–65 × ?		–		49–58 × 22–24		59–62 × 30–32				60–64 × 30–34	
Spicule (L) [a]	**1.5–1.7**	–	1.22–1.83	–	1.22–1.37	–	0.96–1.02	–	1.5–7.5	–	0.64–0.71	–	0.68–0.71	–
Spicule (W) [a]	**16–23**	–	–	–	20–22	–	–	–	10–12	–	18	–	14–15	–
Spicular sheath spines	**Present**	–	–	–	Absent	–	Present	–	Present	–	Present	–	Present	–
Spicular sheath (L × W) [a]	**123–433 × 13–16**		–	–	–	–	70.010–0.012	–	0.025	–	–	–		–
# Specimen	**20**	**20**	28	11	–	–	–	–	20		3	7	–	–
Reference	**Present study**		Madsen (1945)		Mettrick (1959)		Mettrick (1959)		Mettrick (1959)		Mettrick (1959)		Mettrick (1959)	
Morphometric characterization	*Capillaria cairina* n. sp.		*C. ovopunctata*		*C. collaris*		*C. phasianina*		*C. anatis*		*C. caudinflata*		*C. anatis*	
Specimen sex	**Male**	**Female**	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	***Cairina moschata***		Blackbirds and starlings		*Odontophorus capueira capueira*		*Phasianus colchicus torquatus*		Domestic fowl		British Domestic fowls		*Anas platyrhynchos*	
Locality	**Soure, Pará, Brazil**		Hertfordshire		Brazil		Brazil		Great Britain		Inglaterra		UK	
Total body (L) [a]	**11.8–15.8**	**16.7–28.6**	6.84–10.3	11.2–14.0	8.3	–	17.82–19.77	26.97–38.76	6.70–13.14	8.11–18.34	8.80–17.60	11.88–25.38	11.99–14.46	15.87–24.69
Maximum body (W) [b]	**36–51**	**38–53**	52	62	66	–	66	70–83	35–58	44–60	33–51	38–62	–	–
Nerve-ring (L) [a,c]	**41–70**	**46–95**	–	–	76	–	84–92	105–130	–	–	–	–	–	–
Muscular esophagus (L) [a]	**256–366**	**335–445**	–	–	206	–	365–522	435–566	–	–	–	–	–	–
Muscular esophagus (W) [a]	**13–15**	**13–20**	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [a,c]	**5.0–6.2**	**5.4–8.0**	–	–	3.82	–	6.87–8.04	7.37–8.78	4.23–5.29	4.23–6.70	4.59–7.05	4.59–7.41	–	–
Vulva (L) [a,c]	–	**5.5–12.0**[e]	–	0.050–0.057[e]	–	–	–	0.042–0.097[e]	–	–	–	–	–	–
Vulvar appendage	–	**Present**	–	Present	–	–	–	–	–	–	–	Present	–	–
Egg (L × W) [a]	**35–48 × 18–22**		57–58 × 27–28		–		46–63 × 21–29		55–62 × 22–29		47–58 × 20–24		53–58 × 24–29	
Spicule (L) [a]	**1.5–1.7**	–	0.91 – 0.94	–	0.96	–	1.74–2.41	–	1.06–1.86	–	0.71–1.25	–	1.40–1.97	–
Spicule (W) [a]	**16–23**	–	3–8	–	17	–	17–29	–	14–22	–	3–5	–	–	–
Spicular sheath spines	**Present**	–	Present	–	Present	–	Present	–	Present	–	Present	–	Present	–
Spicular sheath (L × W) [a]	**123–433 × 13–16**		–	–	17 × 21		? × 25–34		–		–		–	
# Specimen	**20**	**20**	–	–	1	–	5	5	100	100	100	100	20	20
Reference	**Present study**		Mettrick (1959)		Freitas et al. (1959)		Freitas et al. (1959)		Wakelin (1965)		Wakelin (1965)		Wakelin (1965)	

[a]Measurements in millimeters; [b]Measurements in micrometers; [c]Calculated from anterior extremity; [d]Calculated from end esophagus. Abbreviations: L = length. W = width. Note: #: numbers.

Table 2. Continued...

Morphometric characterization	*Capillaria cairina* n. sp.		*C. anatis*		*C. anatis*		*C. longicollis*		*C. nyrocinarum*		*C. spinulosa*		*C. tenuissima*	
Morphometric characterization	*Capillaria cairina* n. sp.		*C. anatis*		*C. anatis*		*C. anatis*		*C. anatis*		*C. anatis*		*C. anatis*	
Specimen sex	**Male**	**Female**	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	***Cairina moschata***		*Perdix perdix*		*Anas acuta*		*Corvus monedula; C. splendens*		Ducks Anatinae		*Gallus gallus domesticus*		Chicken	
Locality	**Soure, Pará, Brazil**		Collection of the London		Delta, USSR		Kashmir, India		North-Western Poland		Kashmir valley		Kagoshima, Japan	
Total body (L) [a]	**11.8–15.8**	**16.7–28.6**	–	19.32	6.70–13.14	8.11 – 18.34	8.20–13.5	9.42–16.50	7.71–10.55	6.83–14.04	10.5–12.9	14.3–18.5	6.42–9.97	7.25–16.58
Maximum body (W) [a]	**36–51**	**38–53**	–	–	35–58	44 – 60	28–60	30–64	35–42	40–50	60–90	51–140	36–64	50–80
Nerve-ring (L) [a,c]	**41–70**	**46–95**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (L) [a]	**256–366**	**335–445**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (W) [a]	**13–15**	**13–20**	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [a,c]	**5.0–6.2**	**5.4–8.0**	–	–	4.23–5.29	4.23 – 6.70	4.46–5.85	4.10–6.05	–	–	4.23 – 6.25	4.12–6.0	3.44–5.06	3.00–6.56
Vulva (L) [a,c]	–	**5.5–12.0[e]**	–	–	–	–	–	4.46–6.12[e]	–	–	–	4.34–4.56[e]	–	0.011 × 0.083[e]
Vulvar appendage	–	**Present**	–	–	–	–	–	–	–	–	–	–	–	–
Egg (L × W) [a]	**35–48 × 18–22**		57 × 26		-		48–57 × 24–27		40–52 × 22–25		51–65 × 25–30		53–63 × 24–34	
Spicule (L) [a]	**1.5–1.7**	–	1.52	–	1.06–1.86	–	0.48–0.65	–	0.63–0.92	–	1.0–1.2	–	0.73–1.21	–
Spicule (W) [a]	**16–23**	–	–	–	–	–	13–24	–	–	–	14–25	–	–	–
Spicular sheath spines	**Present**	–	–	–	Present	–	Present	–	Present	–	Present	–	Present	–
Spicular sheath (L × W) [a]	**123–433 × 13–16**		–	–	–	9 × 10	140–180×?		–	–	–		–	–
# Specimen	**20**	**20**	2	2	10	10	10	20	4	11	28		28	29
Reference	**Present study**		Wakelin (1967)		Baruš et al. (1981)		Dar et al. (2013)		Stapf et al. (2013)		Tanveer et al. (2015)		Tamaru et al. (2015)	
Morphometric characterization	*Capillaria cairina* n. sp.		*C. anatis*		*C. anatis*		*C. spinulosa*		*C. spinulosa*		*C. phasianina*		*C. anatis*	
Specimen sex	**Male**	**Female**	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	***Cairina moschata***		Chicken		Chicken *domesticus*		*Anas platyrhynchos*		*Anser cygnoides*		*Phasianus colchicus versicolor*		*Anas platyrhynchos domesticus*	
Locality	**Soure, Pará, Brazil**		Davao Oriental, Philippines		Indonesia		Surabaya, Indonesia		Surabaya, Indonesia		Kumamoto, Japan		Ukraine	
Total body (L) [a]	**11.8–15.8**	**16.7–28.6**	7.69–14.06	12.61–20.83	10.95–13.97	13.56–14.23	15.00–16.71	24.4	12.34–14.58	10.06–23.61	19.29	19.77	7.20–9.80	9.70–12.50
Maximum body (W) [a]	**36–51**	**38–53**	50–72	60–106	53–65	55–58	55–57	71	38–52	52–73	65	65	44.78–47.21	58.17–61.28
Nerve-ring (L) [a,c]	**41–70**	**46–95**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (L) [a]	**256–366**	**335–445**	–	–	–	–	–	–	–	–	–	–	–	–
Muscular esophagus (W) [a]	**13–15**	**13–20**	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [a,c]	**5.0–6.2**	**5.4–8.0**	3.50–6.69	4.97–6.94	4.76–5.77	5.24–5.48	5.88–6.74	6.65	5.27–5.84	5.18–7.89	5.48	5.44	–	–
Vulva (L) [a,c]	–	**5.5–12.0[e]**	–	0.011–0.117[e]	–	0.023–0.068[e]	–	–	–	0.058–0.427[e]	–	–	–	4.45–6.15
Vulvar appendage	–	**Present**	–	–	–	–	–	–	–	–	–	–	–	Absent
Egg (L × W) [a]	**35–48 × 18–22**		49–66 × 26–37		54–59 × 24–29		45 × 24		40–50 × 21–24		41–49 × 24–25		56.29–61.25 × 29.45–35.41	
Spicule (L) [a]	**1.5–1.7**	–	0.89–1.12	–	0.94–1.15	–	0.65–0.72	–	0.74–0.78	–	2.55	–	1.40–1.64	–
Spicule (W) [a]	**16–23**	–	–	–	–	–	–	–	–	–	–	–	10.20–11.62	–
Spicular sheath spines	**Present**	–	Present	–	–	–	Present	–	Present	–	Present	–	Absent	–
Spicular sheath (L × W) [a]	**123–433 × 13–16**		–	–	–	–	–	–	–	–	–	–	–	
# Specimen	**20**	**20**	21	18	3	2	3	1	3	3	1	1	15	15
Reference	**Present study**		Tamaru et al. (2015)		Sakaguchi et al. (2020)		Sakaguchi et al. (2020)		Sakaguchi et al. (2020)		Sakaguchi et al. (2020)		Yevstafieva et al. (2020)	

[a]Measurements in millimeters; [b]Measurements in micrometers; [c]Calculated from anterior extremity; [d]Calculated from end esophagus. Abbreviations: L = length. W = width. Note: #: numbers.

Sakaguchi et al. (2020) constructed a phylogenetic tree based on the SSU rDNA nucleotide sequences of representative species of the Capillariidae family reported in birds and mammals. All species shown in the tree were grouped according to the genera described by Moravec (1982), confirming that the morphological classification in our study matches the species identified in the phylogenetic analysis of Sakaguchi et al. (2020). Phylogenetic analyses have become a complementary part of studies involving species identification, thus establishing current genetic relationships between capillariid species. This helps elucidate their complex taxonomy and phylogenetic arrangements to corroborate the existing taxonomic classification (Tamaru et al., 2015; Borba et al., 2019; Deng et al., 2021).

The pairwise genetic distance between our isolate and *C. spinulosa* was 6%, this distance is considered high because most distances between capillariid species belonging to the same genus are less than 1%, according to Tamaru et al. (2015), Sakaguchi et al. (2020) and Garbin et al. (2021). Sakaguchi et al. (2020) noted that integrative approaches are highly recommended for identifying capillariids. Thus, in the phylogenetic tree constructed in our study, the species were grouped according to the site of infection, order, and host family. These factors determine the degree of proximity between the *Capillaria* species analyzed in this study.

Conclusion

Through morphological, morphometric, and phylogenetic analyses of the capillariid nematodes in our study, we identified a new species, *Capillaria cairina* n. sp., which parasitizes the large intestine and ceca of *C. moschata*.

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Ethics declaration

Approval was obtained from the research ethics committees to achieve the objectives of this study because no live animals were used in the study. Protocols: 030/2018 (CEUA) and 23084.014807/2018-80 (UFRA).

Conflict of interest

The authors declare that they have no conflict of interest.

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ARTIGO 4

Título: Systematic and parasite-host relationship by *Baruscapillaria appendiculata* in *Phalacrocorax brasilianus* collected from Marajó Island, State of Pará, Brazil

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Systematic and parasite-host relationship by *Baruscapillaria appendiculata* in *Phalacrocorax brasilianus* collected from Marajó Island, State of Pará, Brazil

Sistemática e relação parasito-hospedeiro por *Baruscapillaria appendiculata* em *Phalacrocorax brasilianus* da Ilha de Marajó, Estado do Pará, Brasil

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Abstract

The genus *Baruscapillaria* Moravec, 1982 has six valid species recorded in birds Phalacrocoracidae, namely *Baruscapillaria appendiculata* Freitas, 1933, *B. spiculata* Freitas, 1933, *B. carbonis* (Dubinin & Dubinina, 1940), *B. jaenschi* (Johnston & Mawson, 1945), *B. phalacrocoraxi* (Borgarenko, 1975) and *B. rudolphii* Moravec, Scholz and Našincová, 1994. Helminthological tests carried out on cormorants of the species *Phalacrocorax brasilianus* (Gmelin), a migratory bird that occurs in the northeast of the State of Pará, Brazil, demonstrate *B. appendiculata* parasitizing the cloaca of these birds, through light microscopy, scanning electron microscopy and molecular biology. These studies allowed a redescription of males and females of this nematode in these hosts and in this geographical area through integrative taxonomy. The occurrence of lesions in the cloaca caused by this nematode parasite was registered using histological analysis. This is a new geographic report for this nematode.

Keywords: Suliformes, Phalacrocoracidae, Capillariidae, Brazilian Amazon.

Resumo

O gênero *Baruscapillaria* Moravec, 1982 possui seis espécies válidas registradas em aves Phalacrocoracidae, sendo *Baruscapillaria appendiculata* Freitas, 1933, *B. spiculata* Freitas, 1933, *B. carbonis* (Dubinin & Dubinina, 1940), *B. jaenschi* (Johnston & Mawson, 1945), *B. phalacrocoraxi* (Borgarenko, 1975) e *B. rudolphii* Moravec, Scholz & Našincová, 1994. Exames helmintológicos realizados em mergulhões da espécie *Phalacrocorax brasilianus* (Gmelin), aves migratórias que ocorrem no nordeste do Estado do Pará, Brasil, demonstram *B. appendiculata* parasitando a cloaca dessas aves, através de microscopia de luz, microscopia eletrônica de varredura e biologia molecular. Estes estudos permitiram uma redescrição de machos e fêmeas deste nematódeo, neste hospedeiro e nesta área geográfica, através da taxonomia integrativa. Foi registrada a ocorrência de lesões na cloaca causadas pelo parasitismo desse nematódeo, por meio de análise histológica, sendo um novo registro geográfico para ele.

Palavras-chave: Suliformes, Phalacrocoracidae, Capillariidae, Amazônia brasileira.

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Introduction

The nematodes of the superfamily Trichinelloidea represent a large group with varied morphological and biological characteristics. Most species parasitize all vertebrate taxa, and affect various organs of the body (Moravec, 2001). Birds of the Phalacrocoracidae family have continental and coastal aquatic habits. They use these environments for reproduction and feeding and have a mainly piscivorous diet. There are few references in the literature regarding the fish species that constitute their diet (Piacentini et al., 2015; Oliveira et al., 2019).

Several studies on *Baruscapillaria* Moravec, 1982 of Phalacrocoracidae birds have been carried out and recorded. These include *Baruscapillaria carbonis* (Dubinin & Dubinina, 1940) in *Phalacrocorax carbo* (Linnaeus) in the Czech Republic, and in *Phalacrocorax brasilianus* (Gmelin) in Chile (Moravec et al., 1994; Frantová, 2001; González-Acuña et al., 2020), *B. jaenschi* (Johnston & Mawson, 1945) in *P. carbo*, *P. sulcirostris* (Brandt), *Microcarbo melanoleucos* (Vieillot), *P. fuscescens* (Vieillot) in Australia, *B. phalacrocoraxi* (Borgarenko, 1975) in *P. pygmeus* (Pallas) in the Asia (Baruš & Sergejeva, 1990b; Johnston & Mawson, 1945), *B. rudolphii* Moravec, Scholz & Našincová, 1994 in *P. carbo* in South Moravia and the Czech Republic (Moravec et al., 1994; Moravec & Scholz, 2016), *B. spiculata* (Freitas, 1933) Moravec, 1982 in *P. brasilianus* in Argentina (Garbin et al., 2021). And in Brazil, Monteiro et al. (2011) identified *B. appendiculata* Freitas, 1933 in *P. brasilianus*.

The goals of this study were therefore to report on *B. appendiculata* parasitizing *P. brasilianus* on Marajó Island, State of Pará, Brazil, and to provide an integrative taxonomic species redescription, bringing together the morphological and morphometric data, using optical and scanning electronic microscopy, and molecular analyses, using the partial 18S rDNA gene. Additionally, we present a histopathological analysis of lesions caused by this capillariid on the cloaca of this bird.

Material and Methods

From 2020 to 2022, ten specimens of *P. brasilianus* were obtained from birds found trapped in fishing nets or trapped in fishing pens in the municipality of Soure (0° 13' 55" S; 48° 26' 58" W), Marajó Island, State of Pará, Brazil. The research has a license from ICMBio/SISBIO nº 74195 and license nº 6309230520 from the Ethics Committee in the use of animals. Only the organs of the digestive tract were sent frozen to the laboratory for a search for parasitic helminths. In the laboratory, the organs were separated and placed in Petri dishes with 0.9% NaCl saline solution and examined individually with the aid of a stereomicroscope (Leica ES2) in search of parasites. The taxonomic classification of nematodes was in accordance with Vicente et al. (1995), Moravec (1982), Moravec (2001), De Ley & Blaxter (2002) and Gibbons (2010). The ecological indices of parasitism were analyzed according to Bush et al. (1997), Bautista-Hernández et al. (2015) and Reiczigel et al. (2019).

The harvested nematodes were washed in 0.9% NaCl, fixed in AFA solution (93 parts of 70% ethyl alcohol, 5 parts of formaldehyde and 2 parts of glacial acetic acid) for 24 hours and then stored in 70% alcohol.

Light microscopy

For light microscopy, nematodes were clarified in 0.5% Aman's Lactophenol solution and observed under a Leica DM2500 microscope with a drawing tube and photographed under a Leica DM2500 microscope with Leica camera system type DFC310 FX with Leica Application Suite Software V4 .4. and stored in glycerin alcohol (70% ethanol with 5% glycerin). Measurements are given in micrometers unless otherwise noted and are given as means followed by ranges in the parentheses.

Scanning electron microscopy

For scanning electron microscopy (SEM), forty-five nematodes were fixed in 3% Glutaraldehyde and washed in 0.2M phosphate buffer solution. Each one was washed for one hour, then post-fixed in 1% Osmium Tetroxide, dehydrated in progressive alcohol for one hour each (50%, 70%, 80%, 90%, 100%), and dried at the CO_2 critical point of, metallized with palladium-gold and observed in a TESCAN scanning electron microscope model VEGA 3 as per Carvalho et al. (2022).

Molecular analysis

For molecular and phylogenetic analyses, 30 nematodes were used. The helminths were extracted from the cloaca and fixed in absolute alcohol. Total DNA was extracted with an Invisorb® Spin Tissue Mini Kit (Invitek

Molecular, Berlin, Germany), following the manufacturer's instructions. The SSU rDNA sequence was amplified with forward primers 18S-E (5'-CCGAATTCGTCGACAACCTGGTTGATCCTGCCAGT-3') and reverse primer 18S-A27 (3'-CCATACAAACGTCCCCGCCTG -5') (Olson & Caira, 1999). The final polymerase chain reaction volume was 25 µL, containing 1 ng of DNA template, 20mM Tris pH 8.4, 50mM KCl, 2mM dNTP (Invitrogen®), 1mM Mg2Cl, 0.5 pmol of each primer and 0.2 units of Taq DNA polymerase (Invitrogen®). The amplification profile consisted of 5 min of initial denaturation at 95 °C, followed by 35 1 min cycles of at 94 °C, 1 min at 60 °C, and 1 min at 72 °C, followed by a final extension of 7 min. at 72 °C to polymerize any molecules that might have become dissociated from the polymerase prior to complete fragment synthesis.

The amplicons were submitted to electrophoresis in 1.5% agarose gel and purified with ExoSAP-ITTM (GE Healthcare, UK) and quantified using Nanodrop equipment (ThermoFisher, CA, USA). The samples were sequenced in the Applied Biosystems™ 3500 Genetic Analyzer (ThermoFisher, CA, USA), generating approximately 700 nucleotides for each sequence. The primers that were used to obtain the amplicons, were also used for sequencing.

The nucleotide sequences obtained from the samples were edited and aligned using the BioEdit software (Hall et al., 2011). After comparison with other sequences available in GenBank (BLAST search), the SSU rDNA sequence was aligned with the sequences of 18 species of capillariids available in GenBank. The database includes sequences from *Trichuris suis* (Schrank, 1788) and *Trichuris muris* (Schrank, 1788), which formed the outgroup for the phylogenetic analyses. The consensus sequence of nucleotides reported in the present study is available in GenBank databases.

Bayesian Inference (BI) and Maximum Likelihood (ML) was implemented using the Markov Chain Monte Carlo (MCMC) phylogenetic tree, implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). This analysis was based on two parallel runs of four simultaneous MCMC searches of five million generations each, with one tree being sampled every 250 generations, after discarding the first 1000 trees. The remaining trees were analyzed with MrBayes to estimate the posterior probability of each node in the phylogenetic reconstruction. As indicated by jModelTest 2.1.9 (Darriba et al., 2012), the BI analysis assumed a TIM3ef + I + G model of nucleotide substitution, with the estimated base frequencies (A = 0.2573, C = 0.2202, G = 0.2821 and T = 0.2404), replacement model (A–C = 0.5953, A–G = 2.2582, A–T = 1.0000, C–G = 0.5953, C–T = 3.3969, G–T = 1.0000) and local variables after a gamma distribution (G = 0.5840), there were 88 models at the 100% confidence interval. Genetic distances were determined for the SSU rDNA sequences of capillariid species in PAUP 4.0 (Swofford, 1998).

Histological processing

Three tissue fragments containing parasites inserted in the cloaca were fixed in 10% formaldehyde, dehydrated in increasing concentrations of 70%-100% ethanol, for 1 hour each and clarified in xylol in two baths, for 30 minutes each. Paraffin infiltration was performed with three successive baths in liquid paraffin for 20 minutes each in an oven at 60 °C followed by inclusion, after which they were sectioned into 5 µm thick sections using a ZEISS HYRAX M25 microtome (Tolosa et al., 2003). They were then stained with Hematoxylin-Eosin and Masson's Trichrome, and the images were obtained using a Leica DM 2500 microscope with a digital camera coupled to a LEICA type DFC310 FX with Leica Application Suite V4.4 software.

Voucher specimens were deposited in the Coleção de Invertebrados do Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil: 5 males (MPEG.PLA 000389), (MPEG.PLA 000390), (MPEG.PLA 000391), (MPEG.PLA 000392), (MPEG.PLA 000393) and 5 females (MPEG.PLA 000394), (MPEG.PLA 000395), (MPEG.PLA 000396), (MPEG.PLA 000397), (MPEG.PLA 000398).

Results

Search data

A total of 142 nematodes were recovered from *P. brasilianus* with a prevalence of 80% (8 infected hosts out of 10 analyzed). This means a prevalence of 80%, mean intensity 17.75, mean abundance 14.2 and range of infection 1 to 45 nematodes per bird. All specimens collected showed characteristics compatible with *B. appendiculata* (Freitas, 1933) Moravec, 1982. The parasites were found embedded in the epithelium of the cloacal mucosa. Below are the results of the taxonomic identification of this nematode, performed using morphological, morphometric, molecular, and phylogenetic analyses, as well as analyses of the histopathology of its parasitism.

Nematoda

Enoplea Inglis, 1983

Trichinellida Hall, 1916

Capillariidae Railliet, 1915

Baruscapillaria Moravec, 1982

Baruscapillaria appendiculata (Freitas, 1933) Moravec, 1982

(Description based on light microscopy and scanning electron microscopy: Figures 1-5)

Long-bodied, threadlike nematodes with transversely striated cuticle. Anterior region containing twelve papillae and a pair of amphids. Oral opening circular in shape. Short, narrow muscular esophagus. Nerve ring located in the initial portion of the muscular esophagus. Stichosome consisting of a single row of 43 elongated stichocytes with distinct transverse rings; markedly large stichocytes nuclei and many nucleoli. Two pseudocoelomate glandular cells present at the esophagus-intestine junction. Two bacillary lateral bands along the body in both males and females.

Male (based on 10 specimens with exposed sheath): Body length 14 mm (11–16); and maximum width at the junction between the esophagus and intestine of 48 (40–70). Length of muscular esophagus 323 (267–370) × 14 (13–17), of stichosome 4.82 mm (1.97–6.34), number of stichocytes about 9 (7–14), stichocytes with distinct transverse rings; large stichocytes nuclei. Length of entire esophagus 5.14 mm (4.90–6.84), representing 39% of body length. Nerve ring situated 66 (43–80) from anterior end. Spicule single, sclerotized, measuring 2.07 mm (1.96–2.29) × 10 (8–12); proximal end of spicule rounded. Aspinous spicular sheath, transverse striations, widely spaced and almost smooth in some regions. Posterior end of body truncated, with two distinct, rounded ventrolateral lobes and a pair of large papillae containing a membrane on each papilla. Terminal cloacal opening. Membranous bursa present.

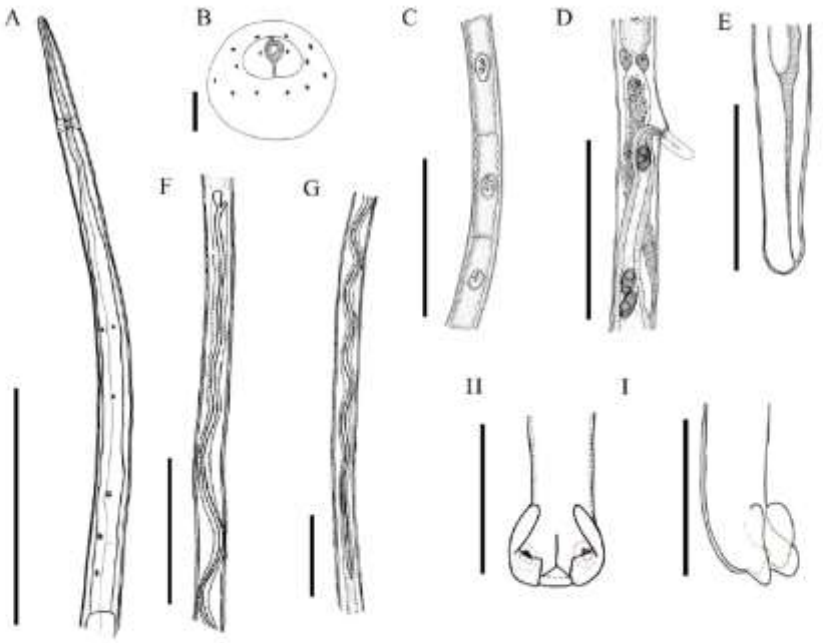

Figure 1. *Baruscapillaria appendiculata* from *Phalacrocorax brasilianus*. A. Anterior end of the female. Scale bar= 20µm. B. Cephalic region containing twelve pairs of cephalic papillae, one pair of amphids, and simple labia (reconstructed from SEM micrograph). Scale bar= 2µm. C. Stichocytes with large nuclei and numerous nucleoli. Scale bar= 30µm. D. Intestinal esophagus junction, shown vulva and vulvar appendix. Scale bar=30µm. E. Female's tail, lateral view. Scale bar= 20µm. F, G. Region of the base of the spicule and the tip of the spicule, respectively. Scale bar= 40µm. H. Male's tail ventral view. Scale bar= 10µm. I. Male's tail, lateral view. Scale bar=10µm.

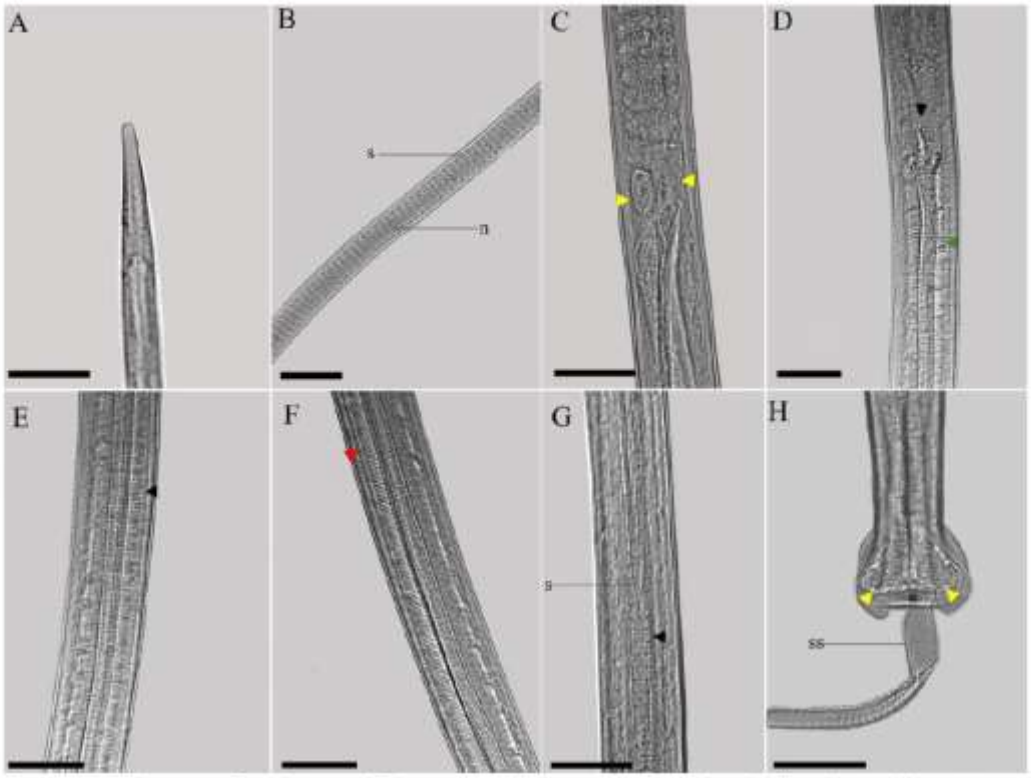

Figure 2. Light microscopy of male *Baruscapillaria appendiculata*. A. Anterior extremity, cephalic region. Scale bar= 50µm. B. Anterior end, note stichocytes (s) and stichocytes nucleus (n). Scale bar= 100µm. C. Intestinal esophagus junction, note the esophageal glands (yellow arrowhead). Scale bar= 50µm. D. Posterior end of male, base of spicule (black arrowhead) and beginning of spicular sheath (green arrowhead) showing transverse striations. Scale bar= 50µm. E. Spicular sheath containing transverse striations, more compact transverse striations can be seen in the proximal section (black arrowhead). Scale bar= 50µm. F. Second cut with transverse striations becoming looser (red arrowhead). Scale bar= 50µm. G. Note tip of spicule (s) and distal segment with transverse striations becoming wider and looser when the spicular sheath is extruded (black arrowhead). Scale bar= 50µm. H. Posterior end, the male's tail can be seen with slightly lateralized caudal lobes (yellow arrowhead) containing a membranous bursa (*) and exposed spicular sheath (ss). Scale bar=50µm.

Female (based on 10 gravid specimens): Body length 25 mm (21–29); and maximum width at the junction between the esophagus and intestine of 62 (53-82). Length of muscular esophagus 452 (348–523) × 18 (17–23), of stichosome 5.68 mm (4.68–7.21), number of stichocytes about 43 (39–46), stichocytes with distinct number of transverse rings; large stichocytes nuclei. Length of entire esophagus 6.15 mm (5.17–7.44), representing 24% of body length. Nerve ring situated 87 (67–130) from anterior end. Distance from the end of the stichocytes to the vulva 130 (58–233). Long vulvar appendage 64 (50–78). Eggs arranged in a single row near the exit of the vagina. Barrel-shaped eggs 43 (42–47) × 21 (20–23), with slightly protruding polar plugs. Egg wall with thick hyaline layer, thin superficial crenate outer layer. Caudal end rounded, anus subterminal.

Molecular characterization and phylogenetic analysis

The rDNA gene sequence obtained for *B. appendiculata* was 1744 bp long and is available on GenBank (accession nº OP828910). A BLAST search revealed that the nucleotide sequences with the greatest similarity were those of *B. spiculata* (accession nº MT068209) described in a grebe from Argentina with 98.83% similarity, and *Aonchotheca putorii* (Rudolphi, 1819) (accession nº MT127177) in a mammal from Japan, with 97.32% similarity.

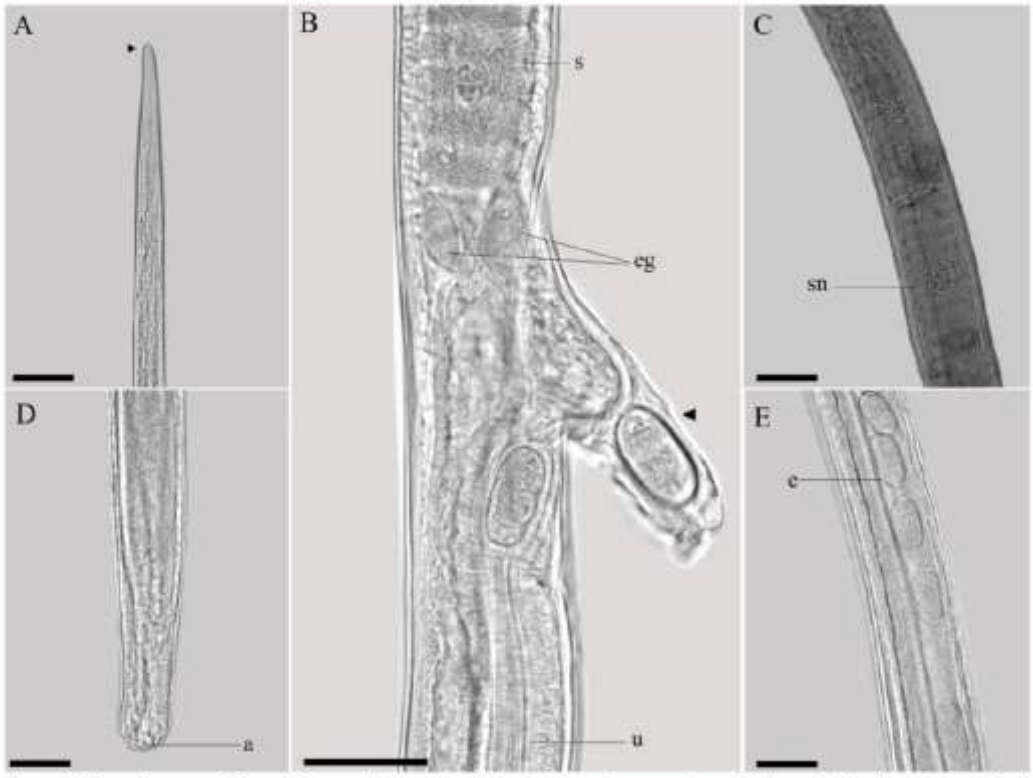

Figure 3. Light microscopy of female *Baruscapillaria appendiculata*. A. Anterior extremity, cephalic region (black arrowhead). Scale bar= 50µm. B. Intestinal esophagus junction, note stichocytes (s), esophageal glands (eg), vulva with vulvar appendage (black arrowhead) containing an egg and uterus (u). Scale bar= 100µm. C. Stichocytes with large nucleus and nucleoli inside (sn). Scale bar= 50µm. D. Posterior end, anus (a). Scale bar= 50µm. E. Embryonated eggs (e) in utero. Scale bar= 50µm.

Molecular characterizations available for *Baruscapillaria* show the type species *B. obsignata* (Madsen, 1945), and the species *B. spiculata* in which the 18S rDNA region was amplified. Therefore, we performed a phylogenetic study to confirm the taxonomic status and generic attribution of our research species. The isolate in the present study showed 98.83% identity with *B. spiculata*. The pairwise genetic distance between the isolates was 0.010 (Table 1). Consequently, these specimens can be considered to belong to the same genus, *Baruscapillaria*.

Phylogenetic analysis based on 18 18S rDNA sequences from Capillariidae species was performed by BI and ML producing well-resolved topologies (Figure 6). Paired DNA analyzes showed genetic distances between the studied taxa ranging from 0.010 to 0.252. The values between *B. appendiculata* (Present study) and *B. spiculata* MT068209 (0.010) were the lowest observed (Table 1).

The isolate from present study formed a well-supported clade A with isolates of *A. putorii*, *A. musimon*, *A.* sp., *A. paranalis* forming the A1 subclade, which are parasites of mammals in Japan and Poland. *B. obsignata* and *B. spiculata*, which are parasites of chickens, pheasants, and ducks in Kagoshima and Yamaguchi in Japan, and cormorants in Argentina formed A2 subclade (Tamaru et al., 2015; Garbin et al., 2021). In the A2 subclade *B. appendiculata* of the present study formed a sister clade with *B. spiculata* having 0.010 of genetic distance (Table 1), although both present a host in common. *B. appendiculata* has morphological characters in the spicular sheath that distinguish it from *B. spiculata*. Still in this subclade *B. obsignata* has 3.9% of genetic distance in relation to present study, although the only similarity is that they all belong to the same genus.

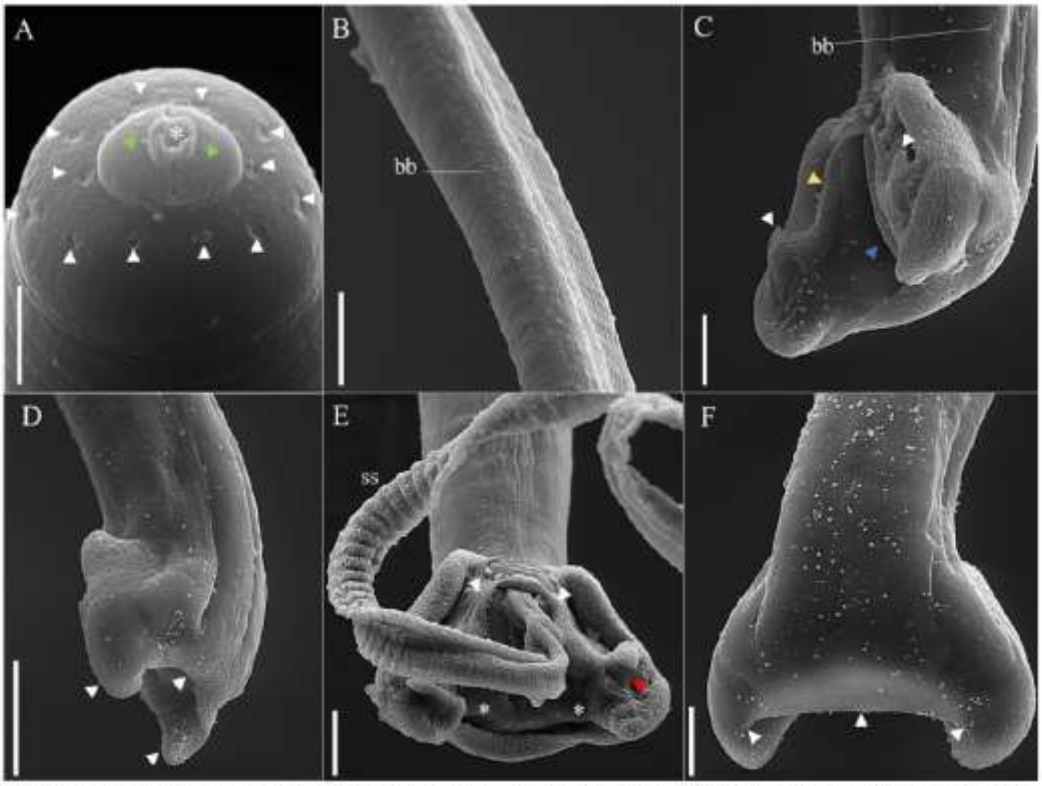

Figure 4. Scanning electron microscopy of male *Baruscapillaria appendiculata* from *Phalacrocorax brasilianus*. A. Button-shaped anterior end, simple lips (*), presence of amphids (green arrowhead) and twelve pairs of cephalic papillae (white arrowhead). Scale bar= 2µm. B. Lateral bacillary band (bb). Scale bar= 20µm. C. Tail, ventrolateral view, observe the bacillary band (bb), papillae (white arrowhead) in each lobe and membrane surrounding the cloaca (yellow arrow) and ventral/medial face of the caudal lobes interconnecting the papillae, cloaca (blue arrowhead). Scale bar= 20µm. D. Posterior extremity, lateral view, membranous bursa (white arrowhead) is observed. Scale bar= 20µm. E. Tail, ventral view, with transverse striations spicular sheath (ss) exposed caudal lobes (*) containing one large papilla each (red arrowhead) where these papillae have a membrane surrounding the cloaca and lobes (white arrowhead). Scale bar= 10µm. F. Dorsal view of the membranous bursa (white arrowhead). Scale bar= 10µm.

Histological analysis

In the fresh tissue samples examined by light microscopy it was possible to observe numerous parasites in the cloaca mucosa and hyperemic areas resulting from this parasitism. In the histological section, males, and pregnant females of *B. appendiculata* were shown with plasmocytes, some lymphocytes and eosinophils, characterizing a moderate inflammatory infiltrate. We observed that the lesion is predominant in the mucous layer where the females are inserted in their tunnels and can transpose the muscularis mucosa and affect the submucosa (Figure 7).

Discussion

According to Moravec (1982), the genus *Baruscapillaria* is diagnosed as having well-developed membranous bursa supported on both sides by one or two small, rounded lobes narrowed at the base; each lobe has a minute projection, usually ventrally folded and a long, well-sclerotized spicule, with a non-spiny spicular sheath. They are parasites of the intestine and stomach of birds and mammals. Freitas (1933a, b) in the original description of *B. appendiculata* described males as having a posterior end provided with two lobes in the form of an "L" involved in a rudimentary caudal bursa and smooth spicular sheath, and as parasites in the large intestine of *P. brasilianus*

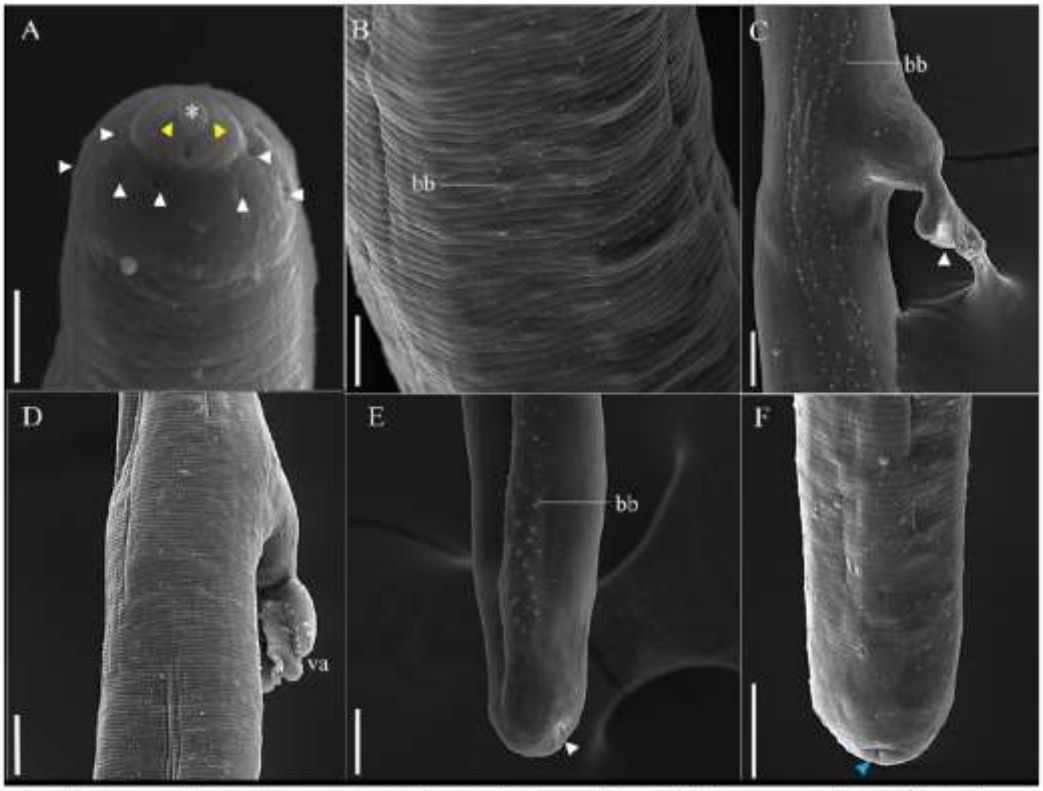

Figure 5. Scanning electron microscopy of female *Baruscapillaria appendiculata* of *Phalacrocorax brasilianus*. A. Button-shaped anterior end, simple lips (*), presence of amphids (yellow arrowhead) and cephalic papillae (white arrowhead). Scale bar= 10µm. B. Lateral bacillary band (bb). Scale bar= 10µm. C. Lateral view of the female's bacillary band (bb), vulva region and well-developed vulvar appendix (white arrowhead). Scale bar= 20µm. D. Dorsal view of the female showing the ventral surface of the vulvar appendix (va). Scale bar: 20µm. E. Lateral view of the female's tail, bacillary band (bb) and anal opening (white arrowhead). Scale bar: 20µm. F. Ventral view of the tail, note the anal opening (blue arrowhead). Scale bar= 20µm.

in Rio de Janeiro. In the description of *B. spiculata*, the male presented a posterior end with four papillae on the tail and the sheath presents spiral striation, distinct throughout most of its extension and parasitizing the cloaca of the same host. In our study, the morphological analysis made it possible to report *B. appendiculata* in *P. brasilianus*, in which the characteristics of the sheath were of major importance for comparison with the species described by Freitas (1933a, b).

Moravec (1982) proposed a new systematic arrangement in the Capillariidae family, reclassifying (according to morphological characters) *Capillaria appendiculata*, originally described by Freitas (1933a), to *B. appendiculata*. Baruš & Sergejeva (1990a) registered a new genus called *Ornithocapillaria*, including only species that parasitized the intestine of birds of the orders Passeriformes, Falconiformes, Strigiformes, and Piciformes. Moravec et al. (2000) used the generic epithet *Ornithocapillaria* to describe specimens found in fish as *O. appendiculata*. Later in his book, Moravec (2001) listed *O. appendiculata* as a synonym of *B. appendiculata*, the most current classification. However, we still find divergences regarding the nomenclature used in different studies (Monteiro et al., 2011; Garbin et al., 2021). The present study corroborates the identification of *B. appendiculata* parasitizing *P. brasilianus*, with the cloaca being the site of infection.

In the most recent study, Garbin et al. (2021) redescribed *B. spiculata* in *P. brasilianus* from Argentina, with a spicular sheath marked by four distinct regularly patterned sections, subterminal cloacal opening and caudal end with a well-developed membranous bursa. In the present study was observed many morphological similarities

Table 1. Pairwise genetic distance data (p-distance) between known capillariid species.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
(1) *Baruscapillaria appendiculata*		-																	
(2) *B. spiculata* MT068209	0.010																		
(3) *B. obsignata* LC425004	0.039	0.044																	
(4) *Aonchotheca paranalis* MF621021	0.058	0.067	0.043																
(5) *Aonchotheca* sp. LC052374	0.067	0.072	0.045	0.024															
(6) *A. putorii* LC052356	0.063	0.070	0.040	0.021	0.016														
(7) *A. musimon* LC052379	0.068	0.076	0.046	0.029	0.022	0.018													
(8) *Eucoleus* sp. LC052381	0.161	0.165	0.133	0.133	0.113	0.115	0.120												
(9) *Eucoleus* sp. LC052382	0.162	0.169	0.140	0.128	0.117	0.118	0.125	0.030											
(10) *E. contortus* LC424996	0.171	0.176	0.145	0.152	0.134	0.132	0.143	0.046	0.047										
(11) *E. dispar* EU004821	0.175	0.178	0.155	0.159	0.145	0.143	0.149	0.049	0.057	0.023									
(12) *E. aerophilus* MW709573	0.188	0.196	0.161	0.163	0.146	0.144	0.152	0.048	0.048	0.020	0.019								
(13) *E. garfiai* LC484432	0.158	0.156	0.137	0.143	0.129	0.128	0.141	0.038	0.039	0.016	0.013	0.008							
(14) *E. perforans* LC424997	0.180	0.187	0.155	0.158	0.140	0.141	0.145	0.049	0.052	0.032	0.034	0.031	0.027						
(15) *Capillaria madseni* LC052347	0.170	0.171	0.157	0.152	0.146	0.154	0.154	0.196	0.201	0.198	0.208	0.216	0.185	0.209					
(16) *C. anatis* LC425001	0.169	0.170	0.145	0.149	0.143	0.144	0.152	0.180	0.190	0.187	0.198	0.196	0.171	0.196	0.096				
(17) *C. pudendotecta* LC052339	0.216	0.219	0.185	0.190	0.169	0.181	0.187	0.213	0.222	0.215	0.237	0.228	0.209	0.241	0.144	0.113			
(18) *C. spinulosa* LC424999	0.220	0.225	0.197	0.193	0.175	0.189	0.192	0.226	0.233	0.227	0.249	0.238	0.221	0.252	0.146	0.133	0.028		
(19) *C. tenuissima* EU004822	0.234	0.244	0.203	0.207	0.197	0.203	0.208	0.242	0.240	0.225	0.239	0.248	0.222	0.249	0.144	0.157	0.185	0.196	-

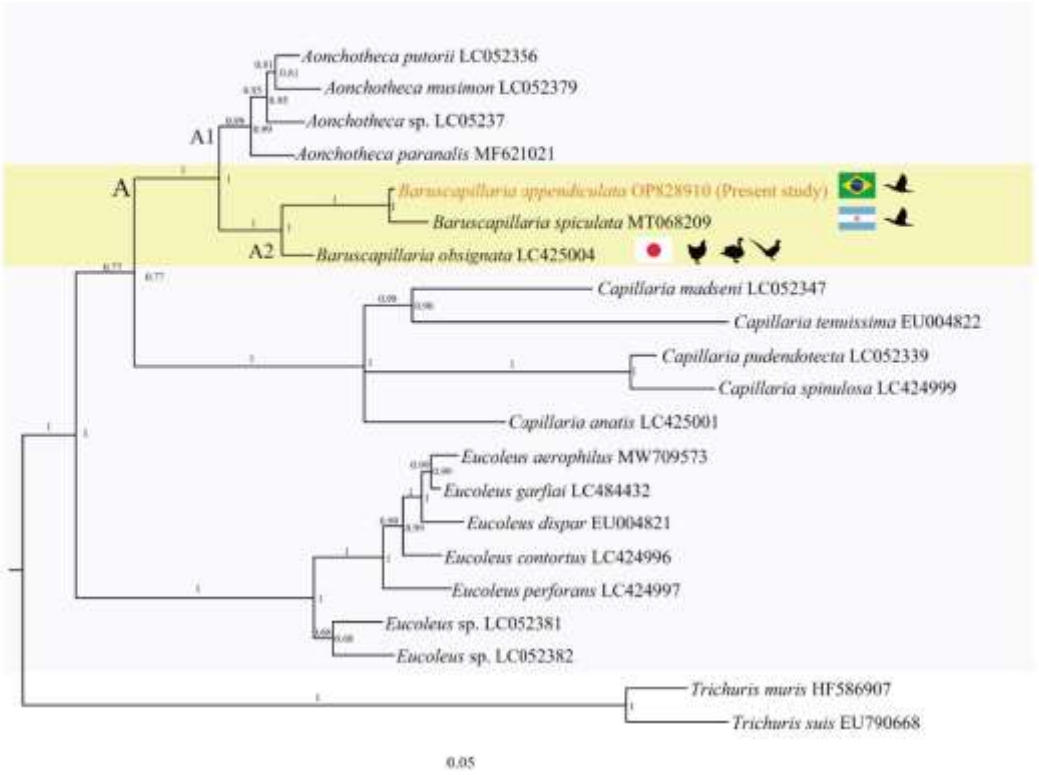

Figure 6. Bayesian phylogenetic tree based on the 18S rDNA sequence obtained from the SSU rDNA analysis of *Baruscapillaria appendiculata* compared to other capillariids. Node numbers represent posterior probability values calculated from BI/bootstrap ML values (> 50%). The scale bar indicates the number of mutations per sequence position. Data are displayed with names of species.

with what was described by the authors above, such as the shape of the caudal end of the male. However, when comparing *B. spiculata* and *B. appendiculata* in the present study significant differences regarding the spicular sheath (Figure 2D-2H). The specimens of *B. appendiculata* deposited by Freitas in 1933 (Freitas, 1933a) at CHIOC were not available for consultation, and those deposited by other researchers from the same period were very damaged, making visualization impossible.

Specimens of *B. appendiculata* capillariids were recorded by Moravec et al. (2000) in *Chirostoma estor* Jordan, 1880 and *Cyprinus carpio* Linnaeus, 1758; however, the authors report that the occurrence in these fish suggests that these nematodes may have been accidentally acquired while the fish were feeding on grebe droppings containing nematodes. As a result, Moravec (2001) in his work rectified *C. estor* and *C. carpio* as accidental hosts of *B. appendiculata* found in the intestine of these fish. This was confirmed in the present study, where we recorded the adult forms in the cloaca of birds, and with pregnant females in all collections of *P. brasilianus*.

Monteiro (2006) recorded *B. appendiculata* parasitizing the large intestine and cloaca of *P. brasilianus* in southern Brazil; however, in the description of these capillariids they observed a non-spiny spicular sheath in males with three distinct regions (reticulate, stellate, and helical), and presence of four bacillary bands, in both. Nonetheless, Garbin et al. (2021) analyzed the specimens described by Monteiro (2006) and concluded that it could be *B. spiculata* and not *B. appendiculata*. In the present study, the specimens were morphologically identified as *B. appendiculata*, which has a reticulate spicular sheath and, as the sheath expands, the reticulate shape becomes more discrete, resembling a smooth sheath, which differs from *B. spiculata* by not have four distinct sections in the spicular sheath, giving a spiral shape as

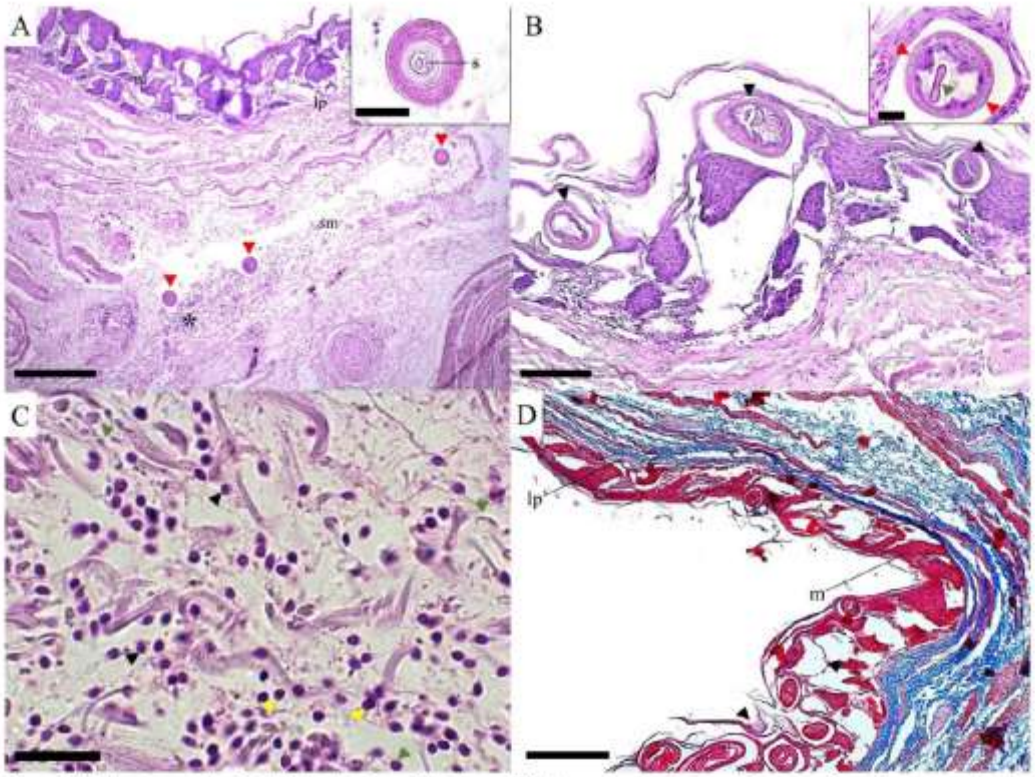

Figure 7. Photomicrograph of histological section of the cloaca of *Phalacrocorax brasilianus*. A. Cross section of the cloaca showing the mucous layer (m), lamina propria (lp) and submucosal layer (sm) with the presence of three cross sections of a *Baruscapillaria appendiculata* male (red arrowhead) and area with inflammatory infiltrate (*) in the submucosal layer (sm). Scale bar = 200µm. In the insert is observed the male's tail where is observed a cross section of the spicule (s). Scale bar = 25µm. Hematoxylin-eosin staining. B. In the mucous layer, is observed cross-sections of female *B. appendiculata* (black arrowhead). Scale bar = 100µm. In the insert there is a cross-section of the female with is observed bacillary (red arrowhead) and egg (green arrowhead) bands. Scale bar = 20µm. Hematoxylin-eosin staining. C. Area of inflammatory infiltrate, lymphocytes (yellow arrowhead), eosinophils (green arrowhead) and plasma cells (black arrowhead). Scale bar = 20µm. Hematoxylin-eosin staining. D. Mucous layer (m) with pregnant females of *B. appendiculata* causing great destruction of the layer (black arrowhead), without affecting the lamina propria (lp). Scale bar = 200µm. Masson's trichrome stain.

originally described by Freitas (1933b) and reaffirmed by Garbin et al. (2021). The comparison of the morphological and morphometric data of the present study with previously published *Baruscapillaria* species is shown in Table 2.

Garbin et al. (2021) state that a complete morphological examination is necessary and must be accompanied by other approaches, including different molecular genetic analyses and evaluation of the geographic distribution of hosts of several species. In the present research, it was necessary to confirm the difference through molecular analysis, where the specimens were identified as the species *B. appendiculata*, since there was a genetic distance of 0.010 in relation to *B. spiculata* and 0.039 in relation to *B. obsignata*.

Sequencing and resequencing more species and large-scale comparative studies can also reveal and correct misidentifications or mislabeled datasets as per Smythe et al. (2019).That is the case with Tamaru et al. (2015) who carried out morphological and molecular characterizations of species of the Capillariidae family, considering the validity of the last classification of the family after the redefinition of Moravec (1982), based on male morphology as the most important morphological characteristic for separating the genera (Moravec, 1982; Gibbons, 2010). This paper provides the first report of the DNA 18S sequence of *B. appendiculata* parasitizing *P. brasilianus*.

Table 2. Morphology and morphometric data comparison of *Baruscapillaria appendiculata* from *Phalacrocorax brasilianus* in State of Pará, Brazil, collected in the present study with *Baruscapillaria* species previously published.

Morphometric characterization	*Baruscapillaria appendiculata*		*B. obsignata*		*B. spiralata*		*B. appendiculata*		*B. obsignata*		*B. obsignata*		*B. pseudobsignata*		*B. phalacrocoracis*		*B. appendiculata*
Specimen sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Female
Host	*Phalacrocorax brasilianus*		chicken and turkey		*P. brasilianus*		*P. brasilianus*		*Cygnus atratus*		Chicken		*P. sulcirostris*, *P. fuscescens*, *P. carbo*, *P. melanoleucos*		*P. pygmeus*		*Cygnus cygnus*
Locality	Pará, Brazil		Small intestine USA		Cloaca Rio de Janeiro, Brazil		Large intestine Rio de Janeiro, Brazil		Small intestine UK		Small intestine UK		? Australia		Bursa Fabricius, cloaca Asia		Intestine Lake Pátzcuaro, Mexico
Total body (L)[a]	11–16	21–29	8.60–10	10–12.70	16	28	–	22.8	12.35–14.79	16.00–20.50	6.90–12.96	8.28–17.28	9.9	7.1–27.4	8.1–15.7	19.8–24.3	21.83
Maximum body (W)[a,c]	40–70	52–82	53	–	70	100	64	89–96	46–49	56–64	42–51	49–67	36	37–41	55–66	88–90	75
Nerve-ring (L)[c,d]	43–80	87–130	–	–	–	–	–	–	–	–	–	–	–	–	–	–	111
Muscular esophagus (L)[c]	267–370	348–523	–	–	–	–	–		–	–	–	–	–	–	–	–	386
Muscular esophagus (W)[c]	13–17	17–23	–	–	–	–	–	–	–	–	–	–	–	–	–	77	–
Total esophagus (W)[a]	4.90–6.84	5.17–7.44	–	–	6–7	6–7	–	6.3	–	–	4.23–6.00	4.96–6.70	–	–	3.9–4.3	5.6–5.9	4.78
Stichosome (W)[a]	1.97–6.34	4.68–7.21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4.39
# Stichocytes	41–52	39–46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	39
Vulva (L)[a]	–	0.058–0.233[e]	–	–	–	0.130[e]	–	0.072[e]	–	–	–	–	–	–	–	0.1[e]	0.081[e]
Eggs mature (LxW)[c]	–	43.27×21.52	–	50–62	–	40–36	–	56–32	–	42–51×22–27	–	44–58×22–28	–	48–50×18–20	–	64–45×22–23	–
Spicule (L)[a]	1.96–2.29	–	1.20	–	2.33	–	1.77	–	1.58–1.78	–	1.09–1.53	–	7	–	3.08–3.30	–	–
Spicule (W)[a]	8–12	–	–	–	–	–	–	–	–	–	–	–	–	–	22–25	–	–
ME/BL (%)	2.41	1.76	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TE/BL (%)	39	24.30	–	–	14.5	–	–	27.6	–	–	–	–	–	–	–	–	–
# Specimen	10	10	2	3	2	2	2 fragmented	not specified	7	8	100	100	1	7	–	–	1
Reference	In this study		Graybill, 1924		Freitas, 1933b		Freitas, 1933a		Wakelin, 1963		Wakelin, 1965		Johnston & Mawson, 1945		Baruš & Sergejeva, 1990a		Moravec et al., 2000

L: length; W: width; ME: muscular esophagus; TE: total esophagus; BL: body length; #: number. [a]Measurements in milimeters. [b]Esophageal-intestinal junction. [c]Measurements in micrometers. [d]Distance from front end. [e]Junction of the intestinal esophagus to the vulva. [f]Fragment. [g]Moravec 2001 book. [h]As recorded by the author. [i]Buenos Aires, Laguna Chis-Chis. [j]Buenos Aires, Laguna San Miguel del Monte.

Table 2. Continued...

Morphometric characterization	*Aonchotheca appendiculata*		*A. appendiculata*		*A. appendiculata*[1]		*A. obsignata*		*A. obsignata*		*A. obsignata*	
Specimen sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	*Phalacrocorax brasilianus*		*Chrysomus icterr*		*P. brachirus*		Chicken		Cygnus olor		Pheasant	
Site of infection	Cloaca		Intestine		Cloaca/large intestine		Small intestine		Small intestine		Small intestine	
Locality	Pará, Brazil		Lake Pátzcuaro, Mexico		Rio Grande do Sul, Brazil		Kagashima, Japan		Yamaguchi, Japan		Kumamoto, Japan	
Total body (L)[a]	11–16	21–29	6.23[f]	14.88–22.13	12.90–16.10	20.75–25.93	5.31–10.61	6.19–10.36	8.58–10.38	10.26–13.67	7.61–8.60	9.00–16.84
Maximum body (W)[a,c]	40–70	50–82	49	65–81	62–65	65–95	28–56	52–64	40–66	57–80	65–89	52–65
Nerve ring (L)[a,d]	62–80	67–100	–	86–153	70–90	–	–	–	–	–	–	–
Muscular esophagus (L)[a]	207–270	248–320	–	315–435	360–477	410–583	–	–	–	–	–	–
Muscular esophagus (W)[a]	12–17	17–23	–	–	–	–	–	–	–	–	–	–
Total esophagus (W)[a]	4.80–6.84	5.17–7.44	–	4.54–6.11	4.60–6.70	4.80–7.00	3.61–6.61	3.94–5.64	6.41–8.42	6.66–8.82	6.10–6.97	6.53–8.71
Stichosome (W)[a]	1.97–6.24	4.88–7.21	–	4.21–6.79	4.60–6.70	4.40–7.00	–	–	–	–	–	–
# Stichocytes	41–52	39–46	–	43–45	45–69	43–45	–	–	–	–	–	–
Vulva (L)[a,e]	–	0.858–0.253[a]	–	0.093–0.175[a]	–	0.087–0.153[a]	–	0.039–0.073[a]	–	0.027–0.110[a]	–	0.035–0.076[a]
Eggs mature (L×W)[a]	–	43.27×21.52	–	57.40×27	–	49.23	–	48–99×24–31	–	–	–	48–26
Spicule (L)[a]	1.90–2.29	–	2.31	–	2.00–3.90	–	0.81–1.26	–	1.03–1.59	–	1.05–1.18	–
Spicule (W)[a]	8–12	–	0.012	–	17–80	–	–	–	–	–	–	–
ME/BL (%)	2.41	1.78	–	–	2.50–3.10	2.90–4.30	–	–	–	–	–	–
TE/BL (%)	30	24.30	–	22–41[a]	30.23	27.26	–	–	–	–	–	–
# Specimen	10	10	5	6	8	6	22	7	9	6	4	5
Reference	In the study		Morazov et al., 2000		Monteiro, 2006		Tanaka et al., 2015				Sakaguchi et al., 2020	

L: length; W: width; ME: muscular esophagus; TE: total esophagus; BL: body length; #: number. [a]Measurements in milimeters. [b]Esophageal-intestinal junction. [c]Measurements in micrometers. [d]Distance from front end. [e]Junction of the intestinal esophagus to the vulva. [f]Fragment. [g]Moravec 2001 book. [1]As recorded by the author. Buenos Aires, Laguna Chis-Chis. Buenos Aires, Laguna San Miguel del Monte.

Table 2. Continued...

Morphometric characterization	*Paracapillaria appendiculata*		*B. obsignata*		*B. obsignata*		*B. obsignata*		*B. spiculata*		*B. spiculata*		*B. bonnemai*	
Specimen sex	Male	Male	Male	Male	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	*Phalacrocorax brasilianus*		Pigeon		Goose		Turkey		*P. brasilianus*		*P. brasilianus*		*Hydropo crinitus australis*	
Site of infection	Cloaca		Small intestine		Small intestine		Small intestine		Cloaca		Cloaca		Intestine	
Locality	Pará, Brazil		Surabaya, Indonesia		Surabaya, Indonesia		Surabaya, Indonesia		Argentina		Argentina		New Zealand	
Total body (L)[a]	11-16	21-30	8.58–11.00	12.67–16.28	7.23–9.67	8.83–12.33	8.05–10.90	12.69–14.63	15.75–17.44	24.00–26.18	12.55–17.25	23.5–27.8	15.2–15.8	23.9–26.3
Maximum body (W)[a,c]	40-70	53-82	40–59	66–76	41–45	69–72	41–47	63–75	50–60	60–80	46–60	65–78	51–55	70–86
Nerve ring (L)[a,d]	42–80	67–130	–	–	–	–	–	–	80–95	95–105	70–85	85–95	–	–
Muscular esophagus (L)[a]	267–370	348–523	–	–	–	–	–	–	295–360	495–670	280–360	390–630	–	–
Muscular esophagus (W)[c]	13–17	17–22	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W)[a]	4.90–6.84	5.17–7.44	3.96–5.24	5.22–6.95	6.68–6.16	6.21–6.20	6.63–5.27	5.19–6.21	6.20–5.95	6.35–7.98	3.85–6.05	5.80–7.50	8.6	7.0–10.3
Isthmus (W)[a]	1.97–6.34	4.68–7.21	–	–	–	–	–	–	3.85–5.64	5.90–7.13	4.27–6.41	6.13–7.32	–	–
# Stichocytes	41–52	39–46	–	–	–	–	–	–	32–44	37–48	34–44	35–45	–	–
Vulva (L)[a,f]	–	0.058–0.233[h]	–	0–0.119[i]	–	0.041–0.094[i]	–	0.076–0.136[i]	–	0.093–0.150[i]	–	0.080–0.138[i]	–	0.107–0.149
Eggs mature (LxW)[c]	–	43.27×21.92	–	46–49×23–28	–	41–49×23–29	–	42–50×25–30	–	46–55×25–27	–	50–56×25–30	–	45–53×25–24
Spicule (L)[a]	1.98–2.29	–	1.25–1.77	–	1.04–1.36	–	1.19–1.32	–	2.20–2.69	–	2.20–2.59	–	1.46–1.49	–
Spicule (W)[c]	8–12	–	–	–	–	–	–	–	–	–	–	–	–	–
ME/BL (%)	2.41	1.76	–	–	–	–	–	–	–	–	–	–	–	–
TE/BL (%)	30	24.30	–	–	–	–	–	–	32.6–39.2	24.8–29.8	28.11–43.42	25.3–30.3	53	31–43
# Specimen	10	10	6	6	4	9	6	6	10	10	8	8	6	6
Reference	In this study		Sakaguchi et al., 2020						Garbin et al., 2021				Presswell & Bennett, 2022	

L: length; W: width; ME: muscular esophagus; TE: total esophagus; BL: body length; #: number. [a]Measurements in milimeters. [b]Esophageal-intestinal junction. [c]Measurements in micrometers. [d]Distance from front end. [e]Junction of the intestinal esophagus to the vulva. [f]Fragment. [g]Moravec 2001 book. [h]As recorded by the author. [i]Buenos Aires, Laguna Chis-Chis. [j]Buenos Aires, Laguna San Miguel del Monte.

In the present study the histopathological analysis of the sections of the cloaca revealed injuries in the mucosal layer, and intense inflammatory infiltrate due to the presence of nematodes, where plasmocytes, lymphocytes and some eosinophils were observed in the muscular layer of the mucosa. That was due to the high parasitic load of capillariids in the cloaca, differing from the types of cellularity present in the inflammatory infiltrate as described by Pinto et al. (2008) recorded *B. obsignata* in *Meleagris gallopavo* (Linnaeus), which caused thickening of the intestinal crypts and villi, together with a mild infiltrated mixed inflammatory picture, in the presence of mononuclear cells and heterophils. That also differs from Carvalho et al. (2021) who described histological changes caused by capillariids of the species *Eucoleus contortus* in the esophagus of the bird *Cairina moschata* (Linnaeus), in which the inflammatory infiltrate predominantly consisted of eosinophils.

Conclusion

This is the first record of *B. appendiculata* parasitizing the cloaca of *P. brasilianus* from Marajó Island, State of Pará, Brazil, based on integrative taxonomy, using morphological, morphometric, and molecular data. The histopathological analysis of the lesions caused by this parasitism was reported.

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Ethics declaration

Approval from research ethics committees was obtained to achieve the objectives of this study, as no live animals were used in the study. Protocols: ICMBio/Sisbio nº 74195 and CEUA/UFRA nº 6309230520.

Conflict of interest

The authors declare no competing interests.

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ARTIGO 5

Título: COMMUNITY OF HELMINTHS PARASITIZING CORMORANTS (SULIFORMES: PHALACROCORACIDAE) FROM THE BRAZILIAN EASTERN AMAZON, PARÁ

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7. COMUNIDADE DE HELMINTOS PARASITANDO CORMORÕES (SULIFORMES: PHALACROCORACIDAE) DA AMAZÔNIA ORIENTAL BRASILEIRA, PARÁ

RESUMO

Este estudo foi realizado no norte do Brasil para determinar a prevalência de helmintos parasitas que infectam aves *Phalacrocorax brasilianus*. Entre junho de 2020 a julho de 2023, parasitas adultos e larvas foram coletados do trato respiratório e gastrointestinal de 30 espécimes dessas aves que morreram em redes de pesca e no curral de pesca na Ilha de Marajó. Os parasitas identificados incluem os nematódeos (*Contracaecum* sp., *Contracaecum australe, Contracaecum rudolphii, C. microcephalum, C. multipapillatum, Syncuaria squamata, Desportesius invaginatus, Tetrameres, Aplectana, Cyathostoma microspiculum, Eucoleus, Baruscapillaria*), trematodeos (*Drepanocephalus spathans, Austrodiplostomum mordax, A. compactum, Hysteromorpha triloba*), cestódeos (*Paradilepis caballeroi*) e Acantocéfalos (*Andracantha* sp., *Southwellina hispida* e *S. macracanthus*). O nível geral de infecção foi de 90% (27/30) e os helmintos mais presentes foram os nematódeos 90% (27/30), seguidos dos Acantocéfalos 66,66% (20/30). Esses dados aumentam o conhecimento sobre helmintos em comorões que migram para ilha de Marajó.

Palavras–chave: parasitos, Phalacrocoracidae, Amazônia brasileira.

ABSTRACT

This study was carried out in northern Brazil to determine the prevalence of helminth parasites that infect birds *Phalacrocorax brasilianus*. Between June 2020 and July 2023, adult parasites and larvae were collected from the respiratory and gastrointestinal tracts of 30 specimens of these birds that died in fishing nets and in the fishing corral on Ilha de Marajó. Identified parasites include nematodes (*Contracaecum* sp., *Contracaecum australe*, *Contracaecum rudolphii, C. microcephalum, C. multipapilllatum*, *Syncuaria squamata*, *Desportesius invaginatus, Tetrameres* sp., *Aplectana, Cyathostoma microspiculum*, *Eucoleus*, *Baruscapillaria*), trematode (*Drepanocephalus spathans, Austrodiplostomum mordax, A. compactum, Hysteromorpha triloba*), Cestodes (*Paradilepis caballeroi*) and Acanthocephalans

(*Andracantha* sp., *Southwellina hispida* and *S. macracanthus*). The overall level of infection was 90% (27/30) and the most common helminths were nematodes 90% (27/30), followed by acanthocephalans 66.66% (20/30). These data increase the knowledge about helminths in cormorants that migrate to Marajó Island.

Keywords: parasites, Phalacrocoracidae, Brazilian Amazon.

7.1.Introdução

Em estudos sobre a diversidade parasitária de *Phalacrocorax brasilianus*, Monteiro *et al*. (2011), realizaram análise da estrutura das comunidades de parasitos nessas aves, encontrando 20 espécies, dessa forma tem despertado interesse devido à ampla distribuição geográfica do gênero *Phalacrocorax* Brisson, 1760.

Os estudos nas áreas onde são encontradas essas aves, revelaram uma helmintofauna extremamente rica, sendo reflexo de um ambiente complexo, rico em invertebrados e vertebrados que atuam como hospedeiro intermediário nos ciclos biológicos para as diferentes espécies de parasitos (Monteiro, 2006). *P. brasilianus* são importantes agentes de disseminação de parasitas devido ao seu hábito migratório (Kennedy, 1998). Porém no Brasil, a helmintofauna ainda não é bem definida.

O número crescente de aves Phalacrocoracidae em associação com muitas espécies de organismos aquáticos (peixes, caracóis do gênero *Lymnaea* etc.) dentro de um território são uma possível razão para a epizootia causada por espécies de parasitos invasores, gerando a possibilidade de disseminação desses parasitos para outros hospedeiros (Yakovleva *et al*. 2020).

O objetivo desta pesquisa é registrar a ocorrência de helmintos nessas aves e contribuir com dados sobre a biodiversidade parasitária de aves no norte do Brasil.

7.2.Material e Métodos

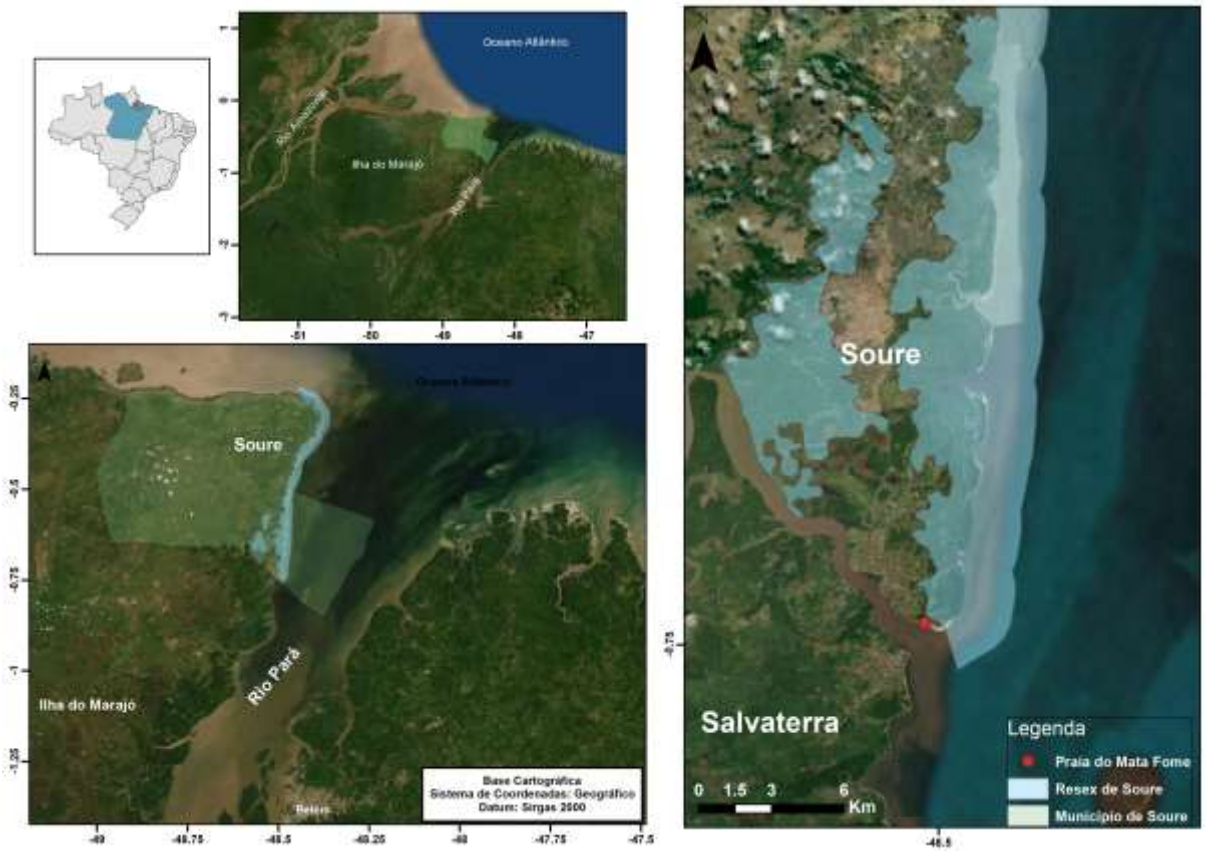
7.2.1. Aspectos éticos

No período de junho 2020 a julho de 2023, sob autorização para atividades com finalidade científica do ICMBio/SISBIO nº 74195, e em conformidade com a Comissão de Ética no Uso de Animais sob protocolo de nº 6309230520.

7.2.2. Aquisição das amostras

Foram adquiridos 30 exemplares (19 machos e 11 fêmeas) de *P. brasilianus* provenientes da Reserva Extrativista Marinha do município de Soure (Figura 1). As amostras foram obtidas das aves encontradas presas em rede de pesca ou presas em currais de domínio de alguns membros da associação de pescadores do município de Soure, estado do Pará. Apenas os órgãos do trato respiratório e digestório foram enviados congelados ao laboratório para pesquisa de helmintos parasitas. No laboratório, os órgãos foram separados e colocados em placas de Petri com solução salina de NaCl 0.9% e examinados individualmente com auxílio de estereomicroscópio (Leica ES2) em busca de parasitas.

Figura 1. Localização geográfica do município de Soure com identificação da RESEX Marinha do município, Ilha de Marajó, Estado do Pará.



7.2.3. Processamento dos helmintos

Os nematódeos colhidos foram lavados em água destilada, fixados e armazenados em solução de A.F.A (93 partes de álcool etílico a 70%, 5 partes de formaldeído e 2 partes de ácido acético glacial). Para microscopia de luz, os nematódeos foram clarificados em solução de Lactofenol de Aman 0.5% e observado em microscópio Leica DM2500 com um tubo de

desenho, após isso, foram armazenados em Álcool + Glicerina (50 partes de álcool etílico a 70% e 50 partes de glicerina pura). Os nematódeos foram fixados em A.F.A. quente (65°C), para evitar contração, e facilitar na análise morfológica.

Os acantocéfalos foram mantidos 24 horas em água destilada, no refrigerador para everterem sua probóscide que é de grande importância taxonômica (Amato & Amato, 2010). Foram comprimidos entre lâmina e lamínula, em uma placa de Petri onde se adiciona o fixador A.F.A., o período de compressão será avaliado de acordo com a espessura do helminto (Oyarzún-Ruiz & González-Acuña, 2020). Trematódeos (exceto os menores) foram comprimidos entre lâmina e lamínula, em uma placa de Petri onde se adicionou o fixador A.F.A., o período de compressão foi avaliado de acordo com a espessura do helminto (Oyarzún-Ruiz & González-Acuña, 2020). Os cestodeos foram colocados em água destilada e levados para geladeira para morrerem com a musculatura relaxada, depois foram fixados em A.F.A. sem compressão (Amato & Amato, 2010).

Os helmintos do Filo Platyhelminthes foram corados em carmim alcoólico, de acordo com Amato e Amato (2010) e Oyarzún-Ruiz e González-Acuña (2020), todos montados individualmente entre lâmina e lamínula permanentes no meio de montagem com Erv-Mount®. Os acantocéfalos foram clarificados com Lactofenol de Aman montados temporariamente entre lâmina e lamínula.

Para estudos morfométricos, os helmintos foram desenhados sob microscópio de luz com câmara clara acoplada (LEICA DM2500), sendo medidos 20 espécimes (10 machos e 10 fêmeas), e os dados morfométricos foram apresentados no formato telegráfico seguindo os padrões usados em estudos taxonômicos. Os helmintos desenhados, posteriormente foram redesenhados com canetas com tinta nanquim e escaneados em alta resolução (600 a 1200dpi). E a obtenção de fotomicrografias foram realizadas com auxílio do microscópio LEICA DM2500 com câmera acoplada LEICA *type* DFC310 FX. As pranchas compostas de desenhos, imagens obtidas com o fotomicroscópio foram utilizando o software *Adobe Photoshop CS*®.

A classificação taxonômica dos nematódeos foi de acordo com Vicente et al. (1995), Moravec (1982), Moravec (2001) e Gibbons (2010).

7.2.4. Índices parasitológicos

Para determinação dos índices ecológicos do parasitismo, esses helmintos passaram por análises por meio de prevalência (%), intensidade média de infecção (IMI) e abundância média, conforme Bush *et al*. (1997), Bautista-Hernández *et al*. (2015) e Reiczigel *et al*. (2019). Os

dados tabulados em planilha de Excel® e comparados com os dados presentes na literatura existente para cada táxon identificado.

7.3.Resultados

Dos 30 hospedeiros analisados 36,66% (n=11) eram pertencentes a animais fêmeas e 63,33% (n=19) a machos. Desses 30 exemplares, em 90% (n=27) foi possível evidenciar o parasitismo. O Filo Nematoda foi o grupo mais representativo ocorrendo em 90% (n=27) das aves; a Classe Trematoda e Cestoda com 10% (n=3) e Acanthocephala 66,66% (n=20) das aves. Os helmintos encontrados e seus respectivos parâmetros estão dispostos na Tabela 1.

Tabela 1 - Localização, prevalência, abundância média e intensidade média dos helmintos encontrados em *Phalacrocorax brasilianus* (n=30) obtidos no município de Soure, Ilha de Marajó, Estado do Pará.

Taxa/ Parasito	**Sitio de infecção**	**Prevalência %**	**Abundância média**	**Intensidade média**
Filo Nematoda				
Família Capillariidae				
Baruscapillaria appendiculata Freitas, 1933; Moravec, Salgado-Maldonado & Osorio-Sarabia, 2000	Cloaca	46,7	11	23,6
Baruscapillaria spiculata (Freitas, 1933*)* Moravec 1982	Intestino, cloaca	23,3	1,1	4,7
Eucoleus contortus (Creplin, 1839*)*	Esôfago	6,7	0,56	8,5
Superfamília Habronematoidea Ivaschkin, 1961				
Família Tetrameridae Travassos, 1914				
Tetrameres sp. Creplin, 1846	Proventrículo	20	1,83	9,16
Família Anisakidae Railliet & Henry, 1912				
Contracaecum sp.	Proventrículo, ventrículo	26,7	0,26	0,72
Contracaecum microcephalum		3,3	0,03	1
Contracaecum rudolphii		43,3	0,5	1,15
Contracaecum multipapillatum		6,7	0,1	1,5
Contracaecum australe Garbin, Mattiucci, Paoletti, González-Acuña & Nascetti, 2011		56,7	1	1,76
Família Acuariidae Railliet, Henry & Sisoff, 1912				
Syncuaria squamata Von Linstow, 1883	ventrículo	6,7	0,26	4
Desportesius invaginatus (Linstow, 1901)		3,3	0,03	1
Família Cosmocercidae Railliet, 1916				
Aplectana sp.	ventrículo	3,3	0,16	5
Família Syngamidae Leiper, 1912				
Cyathostoma microspiculum	Traqueia	26,7	0,63	2,37

Filo Platyhelminthes, Classe Trematoda **Família Diplostomidae**				
Austrodiplostomum mordax Szidat & Nani, 1951	Intestino	6,7	0,06	1
Austrodiplostomum compactum (Lutz, 1928) Dubois, 1970	Intestino	6,7	0,6	9
Hysteromorpha triloba (Rudolphi, 1819) Lutz, 1931	Intestino	3,3	0,03	1
Família Echinostomatidae				
Drepanocephalus spathans Dietz, 1909	Intestino	6,7	0,16	2,5
Família Gorgoderidae	Intestino	3,3	0,03	1
Filo Platyhelminthes, Classe Cestoda **Família Dilepididae Railliet & Henry, 1909**				
Paradilepis caballeroi	Intestino	23,3	1,5	6,42
Filo Acanthocephala Rudolphi, 1808 **Classe Palaeacanthocephala Meyer, 1931** **Família Polymorphidae Meyer, 1931**				
Andracantha sp.	Intestino delgado e grosso	36,7	3,83	4,09
Southwellina hispida (Van Cleave, 1925)	Intestino delgado	23,3	2,96	12,71
Southwellina macracanthus (Ward & Winter, 1952)	Intestino e cecos	6,7	1,5	22,5

Durante as dissecações dos órgãos, a composição de seus alimentos também foi registrada: *Macrobrachium amazonicum* (Heller, 1862), *Batrachoides surinamenses* (Bloch & Schneider, 1801), *Chloroscombrus chrysurus* (Linnaeus, 1766), *Pomadasys* sp. Lacepède, 1802, *Psectrogaster rhomboides* Eigenmann & Eigenmann, 1889, *Eugerres brasilianus* (Cuvier, 1830), *Harttia depressa* Rapp Py-Daniel & Oliveira, 2001, *Lithodora dorsalis* Valenciennes, 1840, *Gobioides* sp. Lacepède, 1800, *Plagioscion squamosissimus* (Heckel, 1840), *Pimelodus* sp.

7.4.Discussão

Nesta pesquisa identificamos 21 espécies de helmintos em *P. brasilianus* oriundos da Ilha de Marajó. Estudos semelhantes foram realizados por Fedynich et al., 1997 e Monteiro et al., 2011 nos quais encontraram uma fauna de endohelmintos com 20 a 21 espécies identificadas. No geral, esses resultados indicam que as comunidades eram semelhantes em estrutura no nível do componente, o que é atribuído aos corvos-marinhos de ambas as lagoas se alimentando da mesma espécie de peixe, pelo que são expostos aos estágios infecciosos de um grupo muito semelhante de espécies de parasitas. As diferenças no nível da infracomunidade

podem ser atribuídas a diferenças no grau de dominância, pois as infracomunidades mais ricas e diversificadas foram as que mais apresentou uma maior uniformidade na abundância de espécies (Violante-González et al., 2011).

A *Baruscapillaria appendiculata* e *B. spiculata* foram encontradas pela primeira vez no Rio de Janeiro, no intestino de *P. brasilianus* por Freitas (1933), após esse período houve redescrição da *B. appendiculata* por Moravec *et al*. (2000; 2001) em um achado no intestino de peixes da região do México. E Garbin et al. (2021) redescreveram *B. spiculata* no mesmo hospedeiro, porém na Argentina. Em nossa pesquisa podemos registrar *B. appendiculata* e *B. spiculata* corroborando assim com os demais estudos.

Os estudos da fauna parasitária do corvo-marinho neotropical na América do Sul, têm sido realizados principalmente no Brasil (Vicente et al., 1996; Monteiro et al., 2011) e Argentina (Drago et al., 2011). González-Acuña et al. (2020) identificaram 12 espécies de parasitos de *P. brasilianus* no Chile entre eles *Hysteromorpha triloba*, *Andracantha* e *Paradilepis caballeroi* em intestino, sendo semelhante ao nosso estudo.

No Brasil, espécimes adultos de *Austrodiplostomum compactum* foram encontrados em *P. brasilianus*, coletados na região Sul do bioma "Campos Sulinos" por Monteiro et al. (2011). Monteiro et al. (2016) registraram adultos de *A. compactum* em *P. brasilianus* no bioma "Cerrado". Em nosso estudo registramos além de *A. compactum*, *A. mordax*, *Drepanocephalus spathans* e *Hysteromorpha triloba*, e um membro da Família Gorgoderidae sem identificação da espécie, semelhante ao estudo de Monteiro (2006).

Espécies de trematódeos pertencentes ao gênero *Drepanocephalus* são parasitas intestinais de aves piscívoras, *Phalachrocorax* spp., e são amplamente relatados nas Américas (PINTO *et al*., 2015).

Em nossa pesquisa, as espécies de peixes encontradas no estômago de *P. brasilianus* diferem de Moravec e Scholz (2016) onde registraram *Cyprinus carpio* Linnaeus, *Tinca tinca* (Linnaeus) e ocasionalmente *Hypophthalmichthys molitrix* (Valenciennes) e pequenos ciprinídeos brancos, *Rutilus rutilus* em *Phalacrocorax* da República Tcheca. Os parasitas digenéticos adultos registrados em aves, e as larvas metacercárias em peixes conforme Monteiro et al. (2016). Portanto em nossa pesquisa o registro das espécies de peixes que compuseram a dieta dos hospedeiros foi importante, pois os peixes podem conter a metacercária.

Os espécimes de cestódeos encontrados no intestino das aves de nossa pesquisa, foram identificados como sendo da espécie *Paradilepis caballeroi* o qual possui 24 ganchos em seu

rostelo, corroborando com Monteiro et al. (2006;2011). A fauna hemintológica de *P. brasilianus* desta pesquisa possui espécies e gêneros de helmintos, que podem ser considerados típicos de aves do gênero *Phalacrocorax* no continente americano, como os nematódeos *Eucoleus contortus*, *Baruscapillaria* sp., *Tetrameres* sp., *Contracaecum rudolphii*, *Syuncaria squamata* e o acantocéfalo do gênero *Andracantha* sp. registrados por estes autores.

O gênero *Aplectana* Railliet & Henry 1916 compreende 51 espécies (Bursey *et al*., 2012; Sou e Nandi, 2015), das quais 27 são conhecidas na América Central e a América do Sul (BURSEY *et al*., 2011; Falcón-Ordaz *et al*., 2014). Esses nematódeos são parasitas intestinais de anfíbios e répteis (Baker, 1987). Em nossa pesquisa registramos a ocorrência deste gênero nas aves.

Por serem animais sentinelas, as aves marinhas apresentam um vasto universo para estudos ecológicos e epidemiológicos. Além disso, os parasitas são componentes estratégicos dos ecossistemas e desempenham papéis importantes por fazerem parte do ciclo evolutivo de muitas espécies de invertebrados e vertebrados terrestres e marinhos (Matos et al., 2020).

7.5.Conclusão

Pela primeira vez foram registradas nos *P. brasilianus* do Brasil as espécies *Baruscapillaria spiculata*, *Cyathostoma microspiculum*, *Aplectana* sp., *Desportesius invaginatus*, *Contracaecum microcephalum*, *Contracaecum rudolphii*, *Contracaecum multipapillatum, Contracaecum australe*. Essa pesquisa auxilia no estudo sobre a dieta das aves que migram para a Ilha de Marajó no Brasil.

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8. CONSIDERAÇÕES FINAIS

Levando em consideração a avaliação da helmintofauna de duas aves, *C. moschata domestica* (ave doméstica) e *P. brasilianus* (ave silvestre), nos deparamos com uma grande diversidade de helmintos. Comparativamente, nessas aves, registramos, nos sítios de infecção como *Eucoleus contortus* no esôfago, e no ventrículo *Contracaecum* spp.; na traqueia Syngamidae sendo comum o achado nestas duas aves. No entanto, *P. brasilianus* por ser uma ave silvestre migratória, apresentou um maior número de parasitos, sendo 17 espécies identificadas e 4 outras somente a nível de gêneros. A comunidade de helmintos em *P. brasilianus* foi caracterizada por alta riqueza de espécies de nematódeos, acantocéfalos e trematódeos, e pequeno número de cestódeos nestas aves.

Ao caracterizarmos a relação parasito-hospedeiro dos sítios de infecção com maior carga parasitária, podemos identificar grandes lesões ocasionadas por *Anisakis* sp., e *Eucoleus contortus* em *C. moschata*. As lesões identificadas em *P. brasilianus* na traqueia, foram ocasionadas por *Cyathostoma microspiculum*; no intestino delgado e cólon ocasionaram lesões pefurantes oriundas da fixação dos acantocéfalos; e na cloaca observamos intenso infiltrado inflamatório ocasionado por *Baruscapillaria appendiculata* e *B. spiculata*, apresentando túneis repletos de ovos. Este estudo foi o primeiro a descrever a lesão histológica associada a infecção por estes parasitos nestas aves.

Assim, identificamos uma nova espécie de *Capillaria* a qual denominamos de *Capillaria cairina* em *C. moschata domestica*, e redescrevemos espécies de helmintos ainda não descritos em *P. brasilianus* como *B. appendiculata* no Brasil. Realizamos a caracterização da relação parasito-hospedeiro prejudiciais a essas aves, adicionando assim, dados sobre a biodiversidade parasitária das duas aves no norte do Brasil.

ANEXOS

Certificado Ceua – *Cairina moschata domestica*

UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Projeto de Pesquisa, intitulado **"Helmintofauna de Patos Domésticos Provenientes da Microrregião do Arari, Ilha de Marajó Pará"**, protocolos **CEUA 030/2018 (CEUA) e 23084.014807/2018-80 (UFRA)**, sob a responsabilidade do professor **Elane Guerreiro Giese**, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, Subfilo Vertebrata (exceto o homem), para fins de pesquisa e/ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS da Universidade Federal Rural da Amazônia em reunião realizada dia 21 de janeiro de 2019.

Vigência do projeto	janeiro 2019/ janeiro2020
Espécie/linhagem	*Cairina moschata domestica*
Número de animais	108 animais
Peso/Idade	3 kg / 8 meses
Sexo	Machos e fêmea
Origem	Os animais são procedências dos municípios de Soure, Salvaterra e Cachoeira do Arari, das criações domésticas de pequenos produtores.

Belém, 04 de janeiro de 2019.

Alex Sandro Schierholt

Prof Dr. Alex Sandro Schierholt
Coordenador CEUA UFRA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA – UFRA
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Autorização ICMBio/SISBIO – *Phalacrocorax brasilianus*

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 74195-4	Data da Emissão: 22/07/2022 10:31:40	Data da Revalidação*: 01/03/2023

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: Elane Guerreiro Giese	CPF: 151.411.602-20
Título do Projeto: TAXONOMIA E FILOGENIA DE HELMINTOS PARASITOS DE Phalacrocorax brasilianus (AVES, PHALACROCORACIDAE) NA ILHA DE MARAJÓ, PARÁ	
Nome da Instituição: Universidade Federal Rural da Amazônia	CNPJ: 05.200.001/0001-01

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta de material biológico	03/2020	03/2022
2	Coleta de material biológico	05/2022	12/2024

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Elaine Lopes de Carvalho	DISCENTE DE PÓS-GRADUAÇÃO	790.736.202-44	Brasileira
2	RICARDO LUIS SOUSA SANTANA	Auxiliar de coleta e processamento de material biológico	007.117.102-95	Brasileira

Ministério do Meio Ambiente - MMA

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Nome da Instituição: Universidade Federal Rural da Amazônia	CNPJ: 05.200.001/0001-01

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Deve-se observar as as recomendações de prevenção contra a COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
3	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989; Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
4	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989; Decreto nº 4.074, de 4 de janeiro de 2002, entre outros)
5	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio nº 03/2014 ou na Instrução Normativa ICMBio nº 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
6	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen
8	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.

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Nome da Instituição: Universidade Federal Rural da Amazônia	CNPJ: 05.200.001/0001-01

Outras ressalvas

1		COINF

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Reserva Extrativista Marinha de Soure	PA	Amazônia	Não	Dentro de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de amostras biológicas in situ	Dentro de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas in situ	Phalacrocorax brasilianus	-

A quantidade prevista só é obrigatória para atividades do tipo "Coleta/transporte de espécimes da fauna silvestre in situ". Essa quantidade abrange uma porção territorial mínima, que pode ser uma Unidade de Conservação Federal ou um Município.

A quantidade significa: por espécie X localidade X ano.

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fezes, Fragmento de tecido/órgão, Sangue

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	Universidade Federal Rural da Amazônia	Laboratório

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0741950420220722

Autorização Ceua – *Phalacrocorax brasilianus*

Comissão de Ética no Uso de Animais CEUA/UFRA

CERTIFICADO

Certificamos que a proposta intitulada "TAXONOMIA E FILOGENIA DE HELMINTOS PARASITOS DE Phalacrocorax brasilianus (Aves: Phalacrocoracidae) NA ILHA DE MARAJÓ, PARÁ", protocolada sob o CEUA nº 6309230520 (ID 000191), sob a responsabilidade de **Elane Guerreiro Giese** *e equipe; ELAINE LOPES DE CARVALHO; RICARDO LUIS SOUSA SANTANA; RAUL HENRIQUE DA SILVA PINHEIRO* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal Rural da Amazônia (CEUA/UFRA) na reunião de 05/08/2020.

We certify that the proposal "TAXONOMY AND PHILOGENY OF HELMINTH PARASITES OF Phalacrocorax brasilianus (Aves: Phalacrocoracidae) IN THE ISLAND OF MARAJÓ, PARÁ", utilizing 10 Birds (males and females), protocol number CEUA 6309230520 (ID 000191), under the responsibility of **Elane Guerreiro Giese** *and team; ELAINE LOPES DE CARVALHO; RICARDO LUIS SOUSA SANTANA; RAUL HENRIQUE DA SILVA PINHEIRO* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal Rural University of Amazonia (CEUA/UFRA) in the meeting of 08/05/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2020 a 07/2023 Área: Parasitologia

Origem:	Animais provenientes de doação espontânea						
Espécie:	Aves	sexo:	Machos e Fêmeas	idade:	1 a 20 anos	N:	10
Linhagem:	não se aplica			Peso:	500 a 3000 g		

Local do experimento: LABORATÓRIO DE HISTOLOGIA E EMBRIOLOGIA (LHEA)/INSTITUTO DA SAÚDE E PRODUÇÃO ANIMAL/UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA

Belém, 05 de agosto de 2020

Profa. Dra. Natalia Guarino Souza Barbosa
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal Rural da Amazônia

Profa. Dra. Ernestina Ribeiro dos Santos Neta
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal Rural da Amazônia

Registro de necropsia e coleta

UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
INSTITUTO DE SAÚDE E PRODUÇÃO ANIMAL DA AMAZÔNIA
LABORATÁRIO DE HISTOLOGIA E EMBRIOLOGIA ANIMAL

REGISTRO DE NECROPSIA E COLETAS DE AVES	
Espécie/Hospedeiro:	
Nº Licença/CEUA:	Nº registro:
Procedência:	
Data de necropsia:	Data da coleta:
Responsável pela necropsia:	
Responsável pela coleta:	
Sexo: ♀ ( ) ♂ ( )	Idade: juvenil ( ) Subadulto ( ) Adulto ( )

	Filo/Classe	**Olhos**	**Boca/ Fossas nasais**	**Esôfago**	**Papo**	**Proventrículo**	**Ventrículo**	**Jejuno**	**Íleo**	**Cecos**	**Cloaca**	**Ovário/Oviduto/Testículos**	**Bursa de Fabricius**	**Rins**	**Traqueia**	**Pulmão**	**Pâncreas**	**Ductos biliares/Ves. biliar**
	Nematoda																	
Platyhelminthes	**Cestoda**																	
Platyhelminthes	**Trematoda**																	
	Acantocephala																	

Observações:

ARTIGO 1

Título: Morphological and molecular characterization of *Contracaecum australe* (Nematoda: Anisakidae) parasitizing *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) on the north coast of Brazil

Autores: SANTANA, R. L. S.; **CARVALHO, E. L.**; NETO, J. L. S.; SILVA, M. V. O.; PINHEIRO, R. H. S.; GONÇALVES, E. C.; GIESE, E. G.

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Original Article

Morphological and molecular characterization of *Contracaecum australe* (Nematoda: Anisakidae) parasitizing *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) on the north coast of Brazil

Caracterização morfológica e molecular de *Contracaecum australe* (Nematoda: Anisakidae) parasitando *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) no litoral norte do Brasil

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Abstract

For the first time in Brazil, *Contracaecum australe* is recorded parasitizing *Phalacrocorax brasilianus* (Aves, Suliformes, Phalacrocoracidae) from the Marine Extractive Reserve of Soure on Marajó Island, Brazilian Amazon. Its morphology revealed a body with a transversally striated cuticle, smooth or slightly cleft interlabia, lips with auricles, labial papillae, and conspicuous amphids. In males, the presence of the median papilla on the upper lip of the cloaca and spicules that reach almost half of the body of the parasite. These morphological characters, added to the number and distribution of the pre- and postcloacal papillae of the male specimens, and supported by the molecular phylogeny from the analysis of the ITS-1, 5.8S and ITS-2 genes, allowed the identification of these parasites.

Keywords: Parasites, birds, scanning electron microscopy, nuclear ribosomal DNA, internal transcribed spacers (ITS).

Resumo

Pela primeira vez no Brasil, *Contracaecum australe* é registrado parasitando *Phalacrocorax brasilianus* (Aves, Suliformes, Phalacrocoracidae) da Reserva Extrativista Marinha de Soure na Ilha de Marajó, Amazônia brasileira. Sua morfologia revelou corpo com cutícula estriada transversalmente, interlábios lisos ou levemente fendidos, lábios com aurículas, papilas labiais e anfídeos conspícuos. Nos machos, observa-se a presença da papila mediana no lábio superior da cloaca e espículos que atingem quase a metade do corpo do parasito. Esses caracteres morfológicos, somados ao número e distribuição das papilas pré e pós-cloacais dos espécimes machos, e apoiados pela filogenia molecular a partir da análise dos genes ITS-1, 5.8S e ITS-2, permitiram a identificação desses parasitos.

Palavras-chave: Parasitos, aves, microscopia eletrônica de varredura, DNA ribossomal nuclear, espaçadores transcritos internos (ITS).

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Introduction

Nematodes of the Anisakidae family infect a variety of aquatic organisms at various developmental stages of their life cycle (Anderson, 2000). Among the anisakids, the genus *Contracaecum* Railliet & Henry, 1912, stands out, having been recorded in several locations on the planet (Biolé et al., 2012). They are parasites described in freshwater, brackish, and marine ecosystems that use fish as intermediate and/or paratenic hosts and aquatic mammals and piscivorous birds as definitive hosts (Anderson, 2000; Mattiucci et al., 2008; Mattiucci & Nascetti, 2008; Shamsi et al., 2009a; Garbin et al., 2011; Shamsi, 2014).

For the South American continent, six species of *Contracaecum* parasitizing *Phalacrocorax brasilianus* (Gmelin, 1789) are recorded: *C. caballeroi* Bravo-Hollis, 1939; *C. travassosi* Lent & Freitas, 1948; *C. rudolphii* Hartwich, 1964 (Syn. *C. spiculigerum*); *C. multipapillatum* (Drasche, 1882) Baylis, 1920; *C. australe* Garbin, Mattiucci, Paoletti, González-Acuña and Nascetti, 2011; *C. jorgei* Sardella, Mancini, Salinas, Simões and Luque, 2020 (Torres et al., 2000; Amato et al., 2006; Violante-González et al., 2011, 2015; Garbin et al., 2011; Biolé et al., 2012; González-Acuña et al., 2020; Sardella, et al., 2020).

In terms of Brazilian avifauna, eight species of *Contracaecum* parasitizing different species of birds have been recorded to date: *C. microcephalum* (Rudolphi, 1819) Baylis, 1920; *C. multipapillatum*; *C. granulosum* (Schneider, 1866) Baylis, 1932; *C. crenulatum* Schuurmans-Stekhoven, 1937; *C. caballeroi*; *C. pelagicum* Johnston & Mawson, 1942; *C. plagiaticium* Lent & Freitas, 1948; and *C. rudolphii* (Vicente et al., 1995; 1996; Amato et al., 2006), but only *C. rudolphii* has been recorded parasitizing the Neotropical cormorant in Brazilian territory (Vicente et al., 1996; Amato et al., 2006).

The species *C. australe* was described for the first time in lagoon Santa Elena in Chile, as a parasite of *P. brasilianus*, using morphological and molecular analyses (Garbin et al., 2011). Biolé et al. (2012), recorded the species on the same host in central Argentina and later *P. gaimardi* Lesson & Garnot, 1828 was added as a new host for *C. australe*, the southernmost record of the species in Argentina, thus expanding its geographical distribution and definitive host range (Garbin et al., 2014).

Given the above, this study aims to investigate the nematode parasites of *Phalacrocorax brasilianus* from the Marine Extractive Reserve of Soure, Marajó Island, Pará, employing the perspective of integrative taxonomy.

Material and Methods

From 2020 to 2022, twenty specimens of *P. brasilianus* were obtained from the coastal zone of the municipality of Soure (Marine Extractive Reserve of Soure) on the Marajó Island, Pará, Brazil (Figure 1) (Latitude: -0.742862°, Longitude: -48.507732°). The birds are used as an alternative source of food by fishers in the region, who kindly provided the dead birds that were used in this study. The animals were transported individually in bags and kept refrigerated in isothermal boxes with ice for transport to the Laboratory. In the laboratory, each organ was individualised in a Petri dish containing 0.9% NaCl saline solution and analysed under a stereomicroscope (Leica ES2), cleaned, quantified, and stored in AFA solution (93 parts of 70% ethyl alcohol, 5 parts of formaldehyde, and 2 parts of glacial acetic acid) for morphological studies, and representative specimens were fixed in 70% ethyl alcohol for molecular analyses.

Figure 1. A- Fishing corral located at beach of Mata Fome, Soure Marine Extractive Reserve, the place where the birds were obtained, Marajó Island, State of Pará, Brazil. B- Cormorant stomach containing specimens of *Contracaecum*. (Scale bar: 1 cm).

Light microscopy

For morphology, the nematodes were clarified in Aman Lactophenol 70%, observed under a microscope (LEICA DM 2500) with a digital capture system (LEICA ICC50 HD) and using the Leica Application Suite software version 4.4.0, being drawn under a microscope (LEICA DM 2500) with attached camera lucida, from which photomicrographs and morphological drawings were respectively obtained. For morphometric analyses, fifteen adult males and fifteen gravid females were used, measuring twenty eggs in each female. Measurements are given in millimetres, unless otherwise indicated, and are presented as mean values followed by minimum and maximum values in parentheses. The taxonomic classification of nematodes was performed according to Baruš et al. (1978), Fagerholm (1991), and Garbin et al. (2011). The ecological indices of parasitism were calculated according to Bush et al. (1997) and Bautista-Hernández et al. (2015).

Scanning Electron Microscopy

Twelve nematodes (eight males and four females) were washed in distilled water for 1 hour, post-fixed in 1% Osmium Tetroxide (OSO_4) for 2 hours, and then submitted to dehydration in an increasing series of ethanol from 70% ethanol until 100% for 1 hour in each battery of alcohol, subsequently subjected to the critical point of CO_2, mounted on metallic aluminium supports (stubs), metallized with gold+palladium, and analysed in a scanning electron microscope (VEGA 3 LMU/TESCAN).

Molecular and Phylogenetic Analyses

Four adult nematodes (two males and two females) fixed in 70% ethyl alcohol had a fragment of approximately 5 mm from the central region removed after measuring the total length of the specimens for allocation to molecular analyses by sequencing the regions of the first and second internal spacers transcribed from ribosomal DNA (ITS-1, 5.8S, and ITS-2). The rDNA extraction was performed at the Biomolecular Technology Laboratory of the Federal University of Pará, using a DNA extraction kit (Spin Tissue Mini Kit, Stratec®), following the protocol indicated by the manufacturer.

The ITS-1, 5.8S and ITS-2 regions of the rDNA were amplified using primers NC5 (Forward: 5' - GTA GGT GAA CCT GCG GAA GGA TCA TT - 3') and NC2 (Reverse: 5' - TTA GTT TCT TTT CCT CCG CT - 3') (Zhu et al., 1998).

The final reaction volume was 25 µL, with 2.5 µL of reaction buffer (BUFF), 1 µL of MgCl2, 2 µL of dNTP's, 0.5 µL of each primer, 0.2 µL of Taq-DNA polymerase unit, 17.3 µL of H2O, and 1 µL of extracted DNA. The samples were processed in a thermal cycler (Applied Biosystems™ ProFlex™ PCR System, 3 x 32-well) and subjected to the following conditions: 95°C for 5 minutes followed by 35 cycles at 95°C for 1 minute (denaturation), 56°C for 1 minute (annealing), 72°C for 1 minute (extension), and a final extension at 72°C for 7 minutes. The amplicons were submitted to 1.5% agarose gel electrophoresis. The PCR product was purified with ExoSAP-ITTM, quantified in Nanodrop equipment, and sequenced using NC5 and NC2 primers in AB 3500 Genetic Analyzer equipment, generating approximately 700 nucleotides each.

For the phylogenetic analyses, eighteen species of *Contracaecum* were included in the inner group, and for the outgroup, two taxa were chosen: *C. australe* (ITS-1: HQ389545/ ITS-2: HQ389547), *C. chubutensis* (ITS-1: HQ389546/ ITS-2: HQ389548), *C. fagerholmi* (ITS-1, 5.8S, ITS-2: JF424599), *C. bioccai* (ITS-1, 5.8S, ITS-2: JF424598), *C. eudyptulae* (ITS-1: FM177550/ ITS-2: FM177578), *C. septentrionale* (ITS-1: AJ634784/ ITS-2: AJ634787), *C. variegatum* (ITS-1: FM177531/ ITS-2: FM177541), *C. ogmorhini* (ITS-1: FM177542/ ITS-2: FM177549), *C. bancrofti* (ITS-1, 5.8S, ITS-2: OP782836), *C. overstreeti* (ITS-1, 5.8S, ITS-2: MG515224), *C. microcephalum* (ITS-1: FM177524/ ITS-2: FM177528), *C. multipapillatum* (ITS-1: AM940056/ ITS-2: AM940060), *C. rudolphii* A (ITS-1: JQ071414/ ITS-2: JQ071437), *C. rudolphii* B (ITS-1: JQ071412/ ITS-2: JQ071435), *C. rudolphii* C (ITS-1, 5.8S, ITS-2: FJ822037), *C. rudolphii* D (ITS-1: FM210251/ ITS-2: FM210268), *C. rudolphii* E (ITS-1: FM210257/ ITS-2: FM210271), *C. rudolphii* F (ITS-1, 5.8S, ITS-2: JF424597) and for the outgroup *Strongylus edentatus* (ITS-1, 5.8S, ITS-2: KP693438) e *S. vulgaris* (ITS-1, 5.8S, ITS-2: KP693439).

For phylogenetic reconstruction, alignment was performed with sequences of ribosomal genes available in GenBank having the ITS-1, 5.8S, and ITS-2 regions or the ITS-1 and ITS-2 interval regions using the BioEdit programme (7.2.5). Bayesian inference (BI) analysis were used based on Markov Chain Monte Carlo (MCMC) tree searches performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Two parallel runs of four simultaneous MCMC searches, each with ten million generations, were performed, with a tree being sampled every 500 generations. Results from the first 1000 trees were discarded as burn-in. The remaining trees were analysed in MrBayes to estimate the posterior probability of each node in the phylogenetic reconstruction. The evolutionary model of nucleotide substitution was determined by the Bayesian Information Criterion (BIC) with the JModelTest programme (Posada, 2008), and the most appropriate model chosen was TPM2uf+G. Genetic distances were determined for sequences from the ITS-1, 5.8S, and ITS-2 regions of *Contracaecum* species using PAUP 4.0b (Swofford, 1998).

Results

Morphological data

Examination of the specimens revealed morphological characters that resemble descriptions in the literature (Garbin et al., 2011, 2014; Biolé et al., 2012). Below is the morphological characterization. Morphological and morphometric data for *C. australe* are presented in Table 1.

Ascaridoidea Baird, 1853

Anisakidae Railliet & Henry, 1912

Contracaecum australe Garbin, Mattiucci, Paoletti, González-Acuña and Nascetti, 2011

Based on light and scanning electron microscopy analyses: (Figures 2-5 and Table 1).

General Morphology (based on 42 specimens): Body totally striated transversely. Very evident cephalic collar with a V-shaped lateral region without striations (Figures 2A, 3A, 3B, 4C, 5A, 5B). Three smooth or slightly cleft interlabia reach 4/5 of the length of the lips. Excretory pore is located immediately below the ventral interlabia. Lips longer than wide, each lip bearing three notable medial apical notches (Figures 3B, 4C), with two conspicuous lobed auricles directed laterally. Dorsal lip with two large laterally directed papillae (Figures 4C, 5B). Ventrolateral lips with one large papilla and a very evident amphid, displaced to the lateral line of the body (Figure 4C). Button-shaped deirids located at the level of the nerve ring or immediately posterior (Figures 2A, 4A, 4B, 5A, 5B). Globular ventriculus, posterior ventricular appendix, developed intestinal cecum, 2 to 3 times longer than ventricular appendix (Figures 2B, 5A). Pre-equatorial vulva. Conical tail. Phasmids clearly visible in both males and females (Figure 3C, 3D).

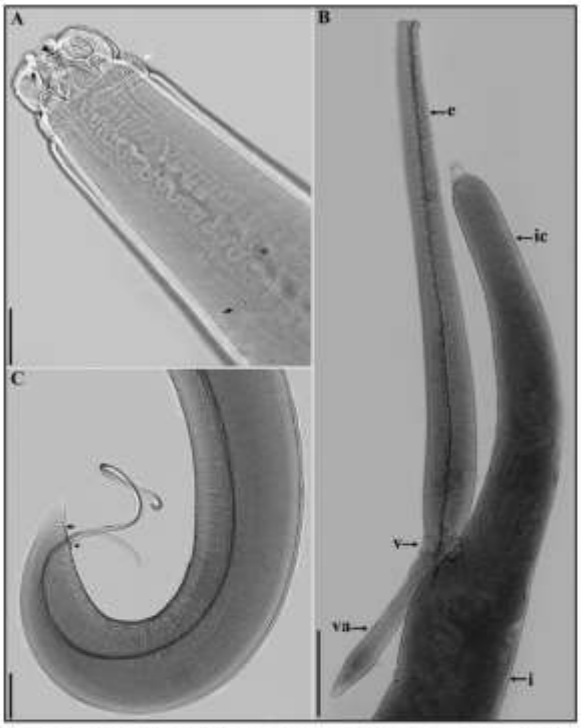

Figure 2. "A-C" Light photomicrographs of *Contracaecum australe*. A- Lateral view of the anterior region where it is possible to observe the ventrolateral lip with a depressed medial apical margin (black head), conspicuous auricle (black arrow), very evident cephalic collar (white arrow), nerve ring (*) and deirids (head of white arrow), scale bar 100 µm. B- Lateral view of part of the dissected digestive system, showing the oesophagus (e), well-developed intestinal cecum (ic), globular ventriculus (v), solid ventricular appendix (va) and intestine (i), scale bar 500 µm. C- Lateral view of the caudal region of a male demonstrating caudal constriction (arrow) and presence of the median papilla on the upper lip of the cloaca (arrowhead), scale bar 300 µm.

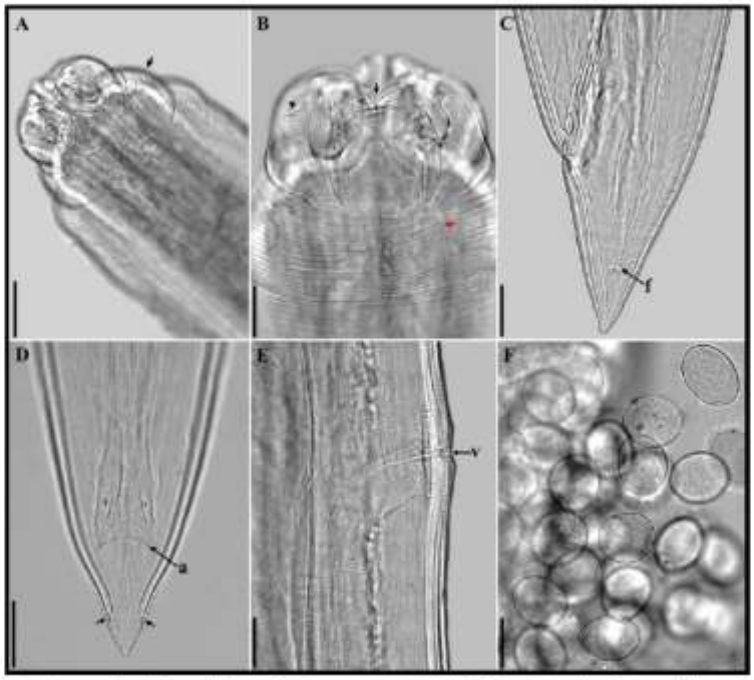

Figure 3. "A-F" Photomicrographs of female from *Contracaecum australe*. In A- Latero-apical view of the cephalic region where interlabia can be observed, conspicuous cephalic collar (black arrow), scale bar 50 µm; B- Lateral view of the anterior region demonstrating the shape of a ventrolateral lip that is longer than it is wide, with a markedly depressed medial apical region (black arrow), interlabia with a bifid apex (arrowhead), cephalic collar with no striations in its lateral region (red arrow), 50 µm scale bar; C- lateral view of the tail of a female showing the phasmid (f), scale bar 100 µm; D- ventral view of the same female tail showing the anus (a) and phasmids (arrows), scale bar 200 µm; E- lateral view demonstrating the shape of the vulva (v), scale bar 100 µm. F- 50 µm scale bar eggs.

Males (based on 15 specimens): Mean body length 25.25 (19.71–28.89). Width at the oesophagus–ventriculus junction 0.64 (0.43–0.83). Body length/body width ratio 39.45 (34.81–45.84). Distance from anterior end to nerve ring and deirids 0.56 (0.50–0.69) and 0.69 (0.51–0.73), respectively. Oesophagus length 3.03 (2.36–3.64). Body length/oesophagus length ratio 8.33 (7.94–8.35). Intestinal cecum length 2.1 (1.5–2.66). Oesophagus length/intestinal cecum length ratio 1.44 (1.37–1.57). Ventriculus length 0.2 (0.11–0.24). Ventricular appendix length 0.804 (0.56–1.03). Oesophagus length/ventricular appendix length ratio 3.77 (3.53–4.21). Spicules subequal, reaching almost half the length of the body, measured 12.18 (9.54–13.91), with rounded distal tip. Body length/spicule length ratio (BL/SL): 2.07 (2.07–2.08). Tail length 0.2 (0.15–0.27). Caudal end conical, having 27–38 pairs of precloacal papillae. Pts zone (= first 25 precloacal transverse striae) including 2 pairs of precloacal papillae. Six pairs of postcloacal papillae are present: 2 paracloacal pairs, 2 subventral pairs, 2 sublateral pairs (Figures 4E, 5C, 5D). Body length/tail length ratio 126.2 (107–131.4). A pair of phasmids between the two pairs of sublateral papillae. Median plaque (median papilla) clearly visible on the anterior border of the cloaca (Figures 4E, 5C). Marked caudal constriction just after the paracloacal papillae (Figures 4E, 5D).

Females (based on 15 gravid females and embryonated eggs): Mean body length 29.66 (22.1–40.94). Width at the oesophagus-ventriculus junction 0.78 (0.59–1.09). Body length/body width ratio 38 (37.4–37.5). Distance from anterior end to nerve ring and deirids 0.64 (0.48–0.76) and 0.68 (0.52–0.83), respectively. Oesophagus length 3.98 (2.89–5.09). Body length/oesophagus length ratio 7.45 (7.65–8.04). Intestinal cecum length 2.69 (2.06–3.46). Oesophagus length/intestinal cecum length ratio 1.48 (7.65–8.04). Ventriculus length 0.29 (0.18–0.40). Ventricular appendix length 0.89 (0.56–1.49). Oesophagus length/ventricular appendix length ratio 4.47 (3.42–5.16). Pre-equatorial vulva, found in the first third of the body. Distance from anterior end to vulva 10.64 (7.59–14.15). Tail length 0.37 (0.29–0.49). Body length/tail length ratio 80.2 (76.2–83.55). One pair of distal phasmids (Figure 3C, 3D). Diameter of embryonated egg 58 (55–60µm) (Figure 3F).

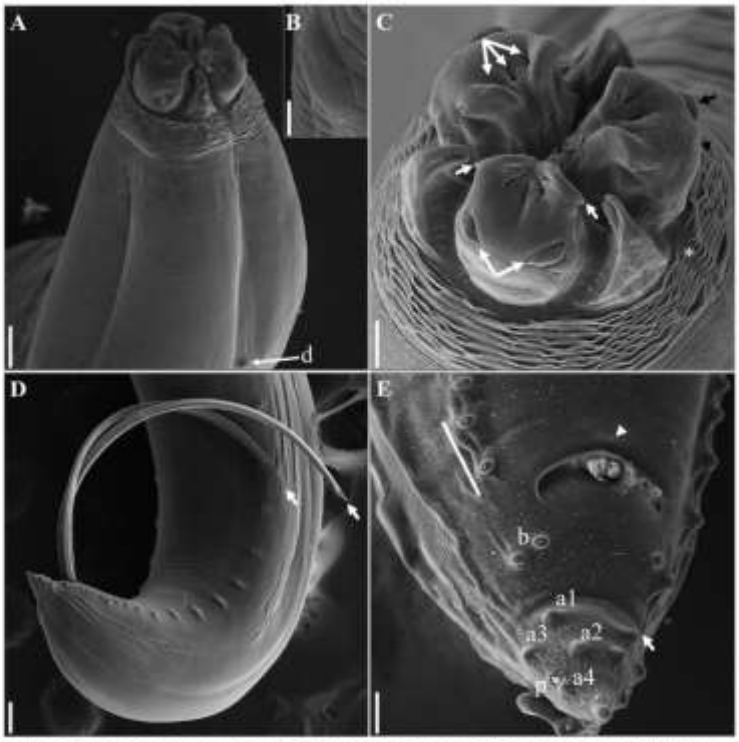

Figure 4. "A-E" Scanning electron microscopy of male *Contracaecum australe*. In A- dorsolateral view showing the presence of the deirids in the form of a button and a smooth surface (d), scale bar 50 µm. B- insert showing the deirid button shape in the largest increase, scale bar 6 µm. C- apical view showing lips with medial apical region with the presence of three clear notches (triple arrow), dorsal lip with the presence of two large papillae (double arrow), and conspicuous auricles (white arrow), interlabia with cleft apex, ventrolateral lips showing the presence of large papilla (black arrow) and amphid (black arrowhead) displaced to the side of the body, well-marked cephalic collar, with absence of striations in the lateral region, V-shaped (*), bar of scale 50 µm. D- lateral view of the tail of a male demonstrating spicules with a rounded free distal tip (white arrows), scale bar 100 µm. E- ventrolateral view of the tail showing the marked caudal constriction (arrow) just after the pairs of paracloacal papillae (b), two pairs of subventral papillae (a1, a2), two pairs of sublateral papillae (a3, a4) and phasmid (p) located between sublateral papillae, presence of median papilla on the upper lip of the cloaca (arrowhead) and pts zone containing 2 papillae, 25 µm scale bar.

Taxonomic Summary

Host: *Phalacrocorax brasilianus* (Gmelin, 1789) (Aves, Phalacrocoracidae).

Location: Soure Marine Extractive Reserve - Marajó Island, State of Pará-Brazil.

Site of infection: Proventriculus and Ventriculus.

Prevalence: 17 infected out of 20 (85%).

Mean intensity and range: 43.7 (7–360).

Molecular data

In our study, the tree topology derived from the phylogenetic analyses inferred from the ITS-1, 5.8S, and ITS-2 intergenic regions of the rDNA of the molecularly analysed specimens (GenBank accession number: OQ397677), demonstrated a 100% correspondence with *C. australe* (Figure 2), grouping it in the same clade and showing it to be distinct from the other species previously genetically characterized and considered for comparison purposes. Parasitic specimens of *P. brasilianus* from Brazil matched previously reported sequences for the ITS-1 and ITS-2 genes of *C. australe* characterised in Chile by Garbin et al. (2011) and deposited in GenBank under accession numbers (ITS-1: HQ389545; ITS-2: HQ389547).

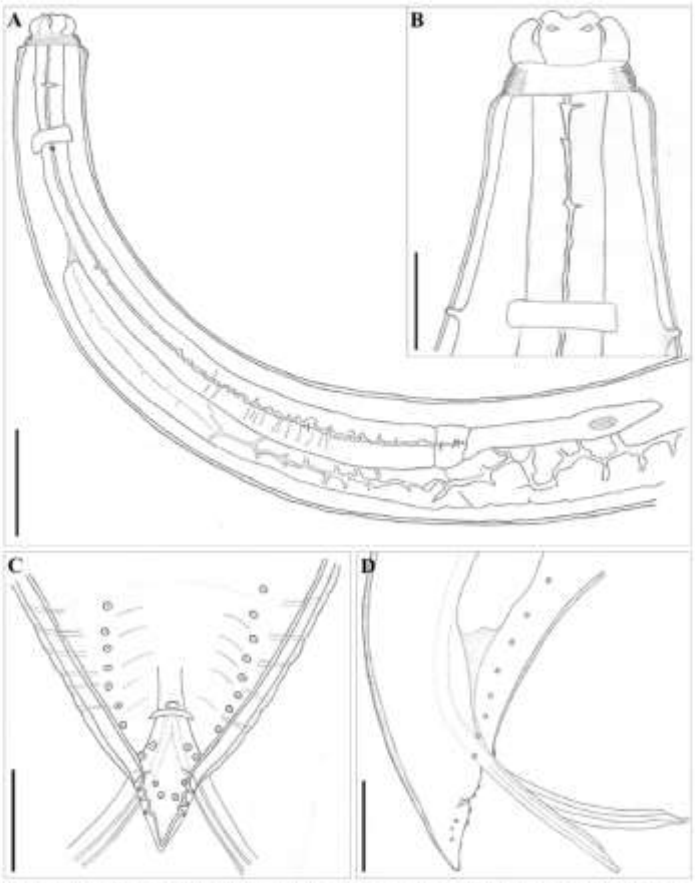

Figure 5. "A-D" Morphological design of *Contracaecum australe*, a parasite of *Phalacrocorax brasilianus* in Northern Brazil. In A- anterior end of body, scale bar 50 µm. B- anterior end, dorsal view, scale bar 20 µm. C- posterior end of male, ventral view, scale bar 20 µm. D- posterior end of male, lateral view, scale bar 20 µm.

A matrix of genetic distances based on the ITS-1, 5.8S, and ITS-2 sequences (2-parameter index from Kimura, 1980) between members grouped according to tree topology is presented in Table 2. The genetic distances between the taxa studied ranged from 0.012 to 0.064. The values between *C. rudolphii* C and *C. ogmorhini* (0.012) were the lowest observed in this study.

Discussion

In this study, nematodes recovered from the proventriculus and ventriculus of *P. brasilianus* on the north coast of the State of Pará, presented morphological characters compatible with *C. australe* (Garbin et al., 2011; 2014; Biolé et al., 2012), making it possible to assign them to this specific taxon, which was found parasitizing *P. brasilianus* from the Marine Extractive Reserve of Soure, Pará-Brazil.

Among the *Contracaecum* species that occur in Brazil, this is the first record of *C. australe* in the national territory. This species was described by Garbin et al. (2011) in the Santa Elena lagoon in Chile as a parasite of *P. brasilianus* using morphological and molecular techniques. Biolé et al. (2012) recorded the species on the same host in the central Argentina region, and later *P. gaimardi* was added as a new host for *C. australe* in the southernmost record of the species in Argentina, thus expanding its geographical distribution and definitive host range (Garbin et al., 2014).

Table 1. Morphometric comparison between *Contracaecum australe* and *C. rudolphii* parasites of Phalacrocoracidae birds in South America.

Characters	*Contracaecum australe*		*Contracaecum australe*		*Contracaecum australe*		*Contracaecum australe*		*Contracaecum rudolphii*	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host Localities Site of infection	*Phalacrocorax brasilianus* Pará-Brazil Proventriculus, Ventriculus		*Phalacrocorax brasilianus* Chile Ventriculus		*Phalacrocorax brasilianus* Argentina Ventriculus		*Phalacrocorax gaimardi* Argentina Ventriculus		*Phalacrocorax brasilianus* Brazil Proventriculus, Ventriculus	
Body [1]	19.71–28.89	22.1–40.94	13.9–28.4	25.44–41.23	19.25–27.37	27–37	15.24–32.33	15.64–36.2	18–31	23–52
Body [2]	0.43–0.83	0.59–1.09	0.64–0.93	0.66–1.16	0.65–1	0.70–0.90	0.49–0.81	0.65–1.05	0.306–0.598	0.50–1.1
Nerve ring [1]	0.50–0.69	0.48–0.76	0.58–0.68	0.50–0.68	0.35–0.39	0.4–0.475	0.43–0.60	0.46–0.60	–	–
Deirids [1]	0.51–0.73	0.52–0.83	0.58–0.79	0.58–0.79	0.35–0.38	0.46–0.55	0.44–0.77	0.49–0.65	–	–
Oesophagus [1]	2.36–3.64	2.89–5.09	2.62–4.6	1.52–3.95	4.12–4.4	3.62–4.5	2.23–3.45	2.56–3.5	2.4–3.8	2.4–5.4
Intestinal cecum [1]	1.5–2.66	2.06–3.46	1.56–3.24	1.3–2.86	3.57–4	3.7–4.25	1.6–2.6	1.66–2.57	2.1–2.9	1.6–3.6
Ventriculus [1]	0.11–0.24	0.18–0.40	0.20–0.38	0.14–0.28	0.1–0.15	0.19–0.23	0.14–0.25	0.2–0.33	–	–
Ventricular appendix [1]	0.56–1.03	0.56–1.49	0.87–1.41	0.57–0.91	0.75–0.85	0.62–0.92	0.73–1.36	0.69–1.33	0.8–1.2	0.6–1.5
Vulva [2]	–	7.59–14.15	–	8.25–10.87	–	8.32–8.45	–	4.70–15.36	–	9.7–21.3
Embryonated egg (µm)	–	55–60	–	63–71	–	47–57	–	50–70	–	91–105
Spicule [1]	9.54–13.91	–	9.6–15.88	–	9.2–10.45	–	7.2–10.44	–	4.5–8.2	–
Precloacal papillae (pairs)	27–38	–	27–32	–	32–40	–	27–43	–	>30[4]	–
Median papilla	1	–	1	–	1	–	–	–	–	–
Postcloacal papillae (pares)	6+1[3]	–	6+1[3]	–	6+1[3]	–	6+1[3]	–	7	–
Tail [1]	0.15–0.27	0.29–0.49	0.18–0.24	0.28–0.58	0.12–0.35	0.12–0.3	0.17–0.25	0.22–0.40	0.14–0.235	0.20–0.60
Ratio BL/BW	34.81–44.6	37.5–37.6	21.72–30.54*	35.54–38.54*	27.37–29.62*	38.57–41.1*	31.10–39.79*	24.06–34.48*	51.8–58.8*	46–47.27*
Ratio BL/EL	7.94–8.35	7.65–8.04	5.31–6.17*	10.44–16.74*	4.67–6.22*	7.46–8.2*	6.83–9.34*	6.11–10.34*	7.5–8.15*	9.58–9.63*
Ratio BL/TL	107–131,4	76.2–83.55	77.22–118.3*	1.17–1.38*	78.2–160.4*	0.98–1.06*	89.65–128.9*	1.36–1.54*	128.6–131.9*	1.5–1.5*
Ratio EL/ICL	1.37–1.57	1.40–1.47	1.42–1.68*	2.67–4.34*	1.1–1.15*	4.89–5.84*	1.33–1.39*	2.63–3.71*	1.14–1.31*	3.6–4*
Ratio EL/VAL	3.53–4.21	3.42–5.16	3.01–3.26*	71.09–90.86*	5.17–5.49*	123.3–225*	2.54–3.05*	71.09–90.5*	3–3.17*	86.67–115*
Ratio BL/SL	2.07–2.08	–	1.45–1.79*	–	2.09–2.62*	–	2.12–3.09*	–	3.78–4*	–
Number of specimens	15	15	10	10	8	4	10	10	30	30
References	Present study		Garbin et al. (2011)		Biolé et al. (2012)		Garbin et al. (2014)		Amato et al. (2006)	

[1]= length; [2]= width; BL/BW= body length/body width ratio; BL/EL= body length/oesophagus length ratio; BL/TL= body length/tail length ratio; EL/ICL= oesophagus length/intestinal cecum length ratio; EL/VAL= oesophagus length/ventricular appendix length ratio; BL/SL= body length/spicule length ratio; [3]= distance from the anterior region to; [4]=more than 30 pairs of precloacal papillae; [3]= +1 pair of phasmids; *ratios calculated with maximum and minimum values provided by the authors.

Table 2. Genetic distance values inferred from the analysis of ITS-1, 5.8S, and ITS-2 sequences between species of *Controcaecum*.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
(1) Study present	-																			
(2) *Controcaecum australe*	0.000	-																		
(3) *Controcaecum chubutensis*	0.021	0.022	-																	
(4) *Controcaecum radiophili* A	0.046	0.048	0.032	-																
(5) *Controcaecum radiophili* B	0.032	0.032	0.024	0.018	-															
(6) *Controcaecum radiophili* C	0.043	0.045	0.029	0.012	0.015	-														
(7) *Controcaecum radiophili* D	0.044	0.046	0.035	0.022	0.018	0.019	-													
(8) *Controcaecum radiophili* E	0.046	0.048	0.033	0.018	0.015	0.015	0.016	-												
(9) *Controcaecum radiophili* F	0.044	0.046	0.033	0.018	0.015	0.015	0.015	0.009	-											
(10) *Controcaecum microcephalum*	0.132	0.132	0.129	0.143	0.125	0.135	0.141	0.135	0.133	-										
(11) *Controcaecum bulgaricum*	0.041	0.043	0.030	0.025	0.030	0.022	0.025	0.025	0.024	0.145	-									
(12) *Controcaecum ogmorhini*	0.043	0.044	0.032	0.016	0.013	0.013	0.013	0.007	0.004	0.131	0.022	-								
(13) *Controcaecum spiculigerum*	0.057	0.059	0.044	0.051	0.045	0.048	0.054	0.049	0.046	0.121	0.047	0.048	-							
(14) *Controcaecum jagerskioldi*	0.067	0.069	0.065	0.074	0.058	0.071	0.076	0.069	0.067	0.143	0.063	0.069	0.068	-						
(15) *Controcaecum kaiccoi*	0.059	0.060	0.055	0.067	0.053	0.062	0.072	0.064	0.063	0.145	0.059	0.062	0.061	0.044	-					
(16) *Controcaecum overstreeti*	0.563	0.562	0.549	0.542	0.555	0.552	0.555	0.557	0.564	0.538	0.555	0.560	0.559	0.593	0.570	-				
(17) *Controcaecum bancrofti*	0.248	0.250	0.246	0.258	0.237	0.253	0.264	0.249	0.256	0.247	0.254	0.249	0.249	0.272	0.257	0.491	-			
(18) *Controcaecum multipapillatum*	0.526	0.525	0.513	0.504	0.518	0.513	0.515	0.517	0.524	0.488	0.518	0.521	0.515	0.547	0.525	0.051	0.495	-		
(19) *Controcaecum variegatum*	0.046	0.048	0.033	0.019	0.013	0.013	0.021	0.016	0.016	0.129	0.024	0.015	0.051	0.067	0.060	0.561	0.259	0.523	-	
(20) *Strongylus edentatus*	200.929	201.366	202.207	196.080	201.449	200.181	195.991	202.939	201.390	244.617	197.807	202.567	210.689	210.485	208.825	212.943	191.009	211.865	202.826	-
(21) *Strongylus vulgaris*	198.510	198.962	198.694	203.888	204.318	202.794	199.211	204.572	203.778	240.223	197.803	204.213	208.147	215.524	212.098	210.092	192.109	203.905	203.002	0.198

Garbin et al. (2011), when describing *C. australe* based on morphological characters considered diagnostic for the species of the genus (*sensu* Hartwich, 1964), such as the length of the spicules, morphology of the distal end of the spicule, and the presence of a slit in the interlabial tip, reported that, a priori, this parasite species of *P. brasilianus* from Chile could be easily attributed to *C. rudolphii lato sensu*. However, after the morphological comparison of the new specimens and due to the presence of characters such as a well-marked constriction in the tail just after the pairs of paracloacal papillae, presence of a median plaque (median papilla), parasites being apparently smaller, more robust and presenting longer spicules than *C. rudolphii* s.l., and supported by phylogenetic analyses of sequences from multiple loci, it was confirmed as a highly supported clade distinct from the rest of the *Contracaecum* taxa considered, thus validating its specific status.

Observing the morphological characteristics of *C. australe* and comparing them to their parasitic congeners of birds, we can see that this species can be easily distinguished from several of them using morphological characters with high diagnostic value (Fagerholm 1988, 1991; Moravec & Scholz, 2016), such as *C. multipapillatum* s.l.; *C. pyripapillatum*; *C. overstreeti*; *C. gibsoni*; *C. bancrofti*; *C. spasskii*; *C. tricuspis*; *C. mexicanum*; *C. ovale*; *C. heardi*; *C. variegatum*, *C. travassosi* that have one or more pairs of double postcloacal caudal papillae. However, sometimes this morphological differentiation is more difficult and requires the use of distinctive diagnostic characters together, such as body length/body width ratio, body length/spike length ratio, and oesophagus length/ventricular appendage length ratio, among others. Garbin et al. (2011, 2014), to differentiate between species, such as between *C. australe* and *C. rudolphii* complex, as these species have a large similarity in the number and pattern of distribution of caudal papillae.

According to Moravec & Scholz (2016), the shape and length of cephalic structures, such as lips and interlabia, the number and arrangement of pre- and postcloacal papillae, as well as the shape of the distal end of the spicules, are taxonomic features that distinguish between *C. rudolphii* and three other congeners parasites of birds, *C. microcephalum*, *C. micropapillatum*, and *C. variegatum*. Currently, the *C. rudolphii* complex has six described cryptic species (*C. rudolphii* A, B, C, D, E, and F) (D'Amelio et al., 2007, 2012; Mattiucci et al., 2008; Shamsi et al., 2009b).

Contracaecum australe can be differentiated from *C. fagerholmi* and *C. rudolphii* F described by D'Amelio et al. (2012), by the length of the spicules that vary from (4.15–4.85 and 5.96–7.30 mm), respectively, as opposed to (9.6–15.88 mm) in *C. australe* described by Garbin et al. (2011), values like those found in the present study (9.54–13.91 mm). However, Biolé et al. (2012), when noting the occurrence of *C. australe* in *P. brasilianus* in Argentina, saw morphometric variations that were considered intraspecific variations, until molecular studies can prove otherwise or corroborate their results. The authors observed a more anterior position of the nerve ring and deirids, a smaller ventriculus and ventricular appendix, a greater number of precloacal papillae, pre-equatorial location of the vulva, and smaller size of the eggs.

Garbin et al. (2014), when adding a new host parasitized by *C. australe* also in Argentina (*P. gaimardi*), pointed out morphometric variations that partially corroborated the findings of Biolé et al. (2012), such as greater amplitude in the number of precloacal papillae, smaller size of the ventriculus and ventricular appendix in male specimens, more pre-equatorial vulva and smaller size of eggs in females. However, the most important morphometric difference occurred in the length of the spicules (7.2–10.44 mm), which were almost a third shorter than those described in specimens found in *P. brasilianus* from Chile (9.6–15.88 mm) and in the present study (9.54–13.91 mm). And as we can observe within the congener species of *Contracaecum* parasites of piscivorous birds, there is a wide range of variation in several metric characters that allow the fitting of multiple species and make it difficult to clearly differentiate.

Contracaecum australe can be differentiated from *C. jorgei*, also recorded in *P. brasilianus* (Syn. *Nannopterum brasilianus*), by the greater length of the spicules (9.54–13.91 vs 2.03–3.63) and the greater number of precloacal papillae (27–38 vs 26), respectively. Furthermore, when describing the species, Sardella et al. (2020) reported the presence of two papillae on the ventrolateral lips, as well as the dorsal lip being longer than the ventrolateral lips, whereas in *C. australe* the lips are the same size and the ventrolateral lips have only one labial papilla and a phasmid in each (Garbin et al., 2011). These results are similar to those found in the present study for the species.

According to Garbin et al. (2011), morphological analyses and differential diagnosis of male specimens of *C. australe* allowed the detection of differences in several characters, including the length of the spicule, the peculiar shape of the male tail, the disposition of the paracloacal papillae, and the depth and shape of the cleft in the interlabium. As for the characters mentioned by the authors, such as caudal constriction after the paracloacal papillae and presence of the median plaque (median papilla), they have not proven to be strong characters for differentiating, for example, between *C. australe* and *C. rudolphii* s.l., since, despite not having been described by some authors, they seem to be clearly present in their illustrations or even photomicrographs. (See, for example, Abollo et al., 2001; Amato et al., 2006; D'Amelio et al., 2012; Moravec & Scholz, 2016), as previously stated by the authors (Garbin et al., 2011).

Garbin et al. (2014) suggested that the specimens described by Amato et al. (2006) as *C. rudolphii* parasites of *P. brasilianus* in Brazil may be *C. australe*, because they share certain morphological characteristics (such as lips, interlips, arrangement, and number of caudal papillae) and the same host. However, when taking into account the length of the spicules and the BL/SL ratio between (*C. rudolphii* 4.5–8.2 mm and 3.8–4 Amato et al., 2006) vs (9.6–15.88 mm and 1.45–1.79 *C. australe* Garbin et al., 2011) and (9.54–13.91 mm and 2.07–2.08 *C. australe* present study), we can see that these species clearly differ and show that these morphological characters seem to be the most consistent ones for differentiating between species, given that, so far, the species of the *C. rudolphii* complex described molecularly and morphologically have smaller spicules than *C. australe*. *C. rudolphii* D 3.90–6.99 mm; *C. rudolphii* E 5.53–6.13 mm Shamsi et al. (2009b); *C. rudolphii* F 5.96–7.3 mm D'Amelio et al. (2012), and higher BL/SL ratios in the species of the *C. rudolphii* complex (*C. rudolphii* D 3.7–3.8; *C. rudolphii* E 4.3–4.4; *C. rudolphii* F 2.5–2.7) than in *C. australe* (1.45–1.79 Garbin et al. 2011; 2.07–2.08 present study). The sister species *C. rudolphii* A, B, and C, despite having been characterised molecularly, have not been morphologically described, and only the length of the spicules is available for these species (D'Amelio et al., 2007; Mattiucci et al., 2008).

We agree with Garbin et al. (2014) when they state the need to review the specimens described by Amato et al. (2006) and, if possible, evaluate them molecularly to complement the morphological diagnosis of these parasites. As previously seen, *P. brasilianus* is a bird capable of harbouring multiple species of *Contracaecum* at the same time (Lent & Freitas, 1948), raising the possibility that this bird presents co-infection with *C. australe* and *C. rudolphii* s.l., and/or more species of the genus in the same individual (Ricardo et al., unpublished data).

For Sardella et al. (2020), the use of molecular techniques is fundamental not only for defining the taxonomic status of these species but also for enabling their recognition. Result observed in the topology of the tree, derived from the inferred phylogenetic analysis of the ITS-1, 5.8S and ITS-2 genic regions of the rDNA of four specimens analysed molecularly in our study, being observed 100% of correspondence with *C. australe* described in Chile (Garbin et al., 2011), if grouping in the same corresponding clade the previously reported sequences for the ITS-1 and ITS-2 genes deposited in GenBank.

In our study, the clade formed in the phylogenetic tree by *C. australe* specimens was distinct from all *Contracaecum* species previously genetically characterized and considered for comparison purposes. In the phylogenetic analyses, it was possible to observe that *C. chubutensis* was the species that was genetically closest to *C. australe*, with a distance of 0.021, but forming two distinct clades. This genetic proximity can be justified by both species being parasites of birds (Phalacrocoracidae) and having the same biogeographical distribution (See Figure 6). However, the smallest genetic distance seen in our study occurred between the species *C. rudolphii* F and *C. ogmorhini* (0.004). Small genetic distances were also observed between *C. ogmorhini* and *C. rudolphii* E (0.007) and between *C. rudolphii* E and *C. rudolphii* F (0.009). Furthermore, the analyses of the data from the ITS-1, 5.8S, and ITS-2 sequences of *C. australe* from Brazil supported its distinction from the cryptic species of the *C. rudolphii complex*, corroborating the results found by Garbin et al. (2011).

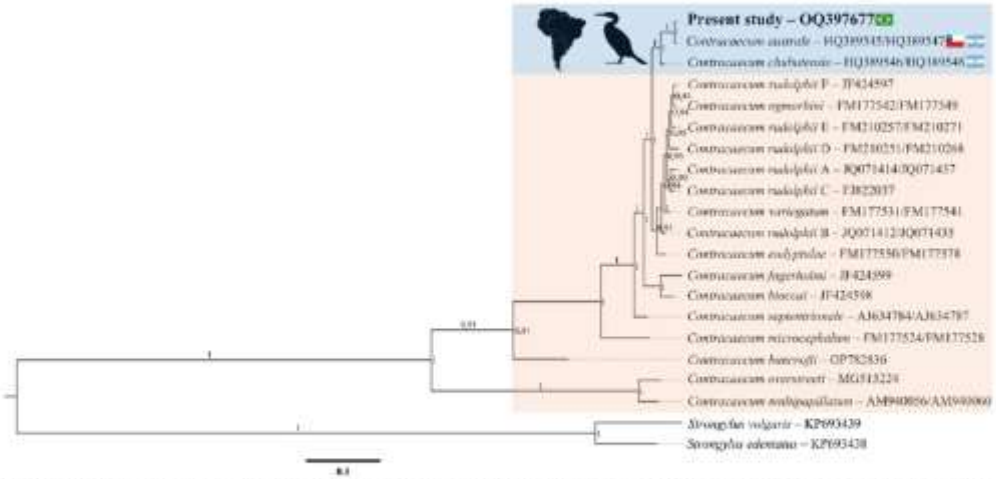

Figure 6. Phylogenetic tree inferred by Bayesian analysis (BI) of *Contracaecum* species, based of Internal Transcribed Spacer ribosomal gene sequences (ITS-1, 5.8S, and ITS-2) and using *Strongylus edentatus* and *Stongylus vulgaris* as outgroups. Node numbers represent posterior probability values recovered by the Bayesian analysis.

Conclusion

Phalacrocorax brasilianus from the north coast of Brazil is the definitive host of *C. australe*; this is the first record of the species in the national territory. In this study, we have expanded the biogeographical distribution of this parasite, in addition to highlighting the need for the application of integrative taxonomy for the characterization of species of *Contracaecum*.

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Ethics declaration

Authorization for activities with a scientific purpose was provided by the System of Authorization and Information on Biodiversity-SISBIO, number: 74195 and under authorization of the Commission for Ethics in the Use of Animals CEUA-UFRA number: 6309230520.

Conflict of interest

The authors declare that they have no conflict of interest.

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ARTIGO 2

Título: Redescription of *Brevimulticaecum baylisi* (Travassos, 1933) Sprent (1979) (Nematoda: Heterocheilidae), a parasite of *Caiman crocodilus* (Crocodylia: Alligatoridae) in the north-eastern Peruvian Amazon

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Original Article

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ABSTRACT

Caiman crocodilus is among the most abundant and widely distributed predators in the Neotropical region. These animals consume prey such as crustaceans, birds, small mammals, reptiles, amphibians, and fish, which can carry infective larval forms of nematodes. *Brevimulticaecum* has few studies on its morphology available, lacking detailed images. Therefore, the aim of this study was to redescribe *Brevimulticaecum baylisi*, stomach parasite of *Caiman crocodilus*, from subsistence hunting in the Yavari-Mirin River, Peruvian Amazon, using light and scanning electron microscopy. Four caimans were analyzed, and, macroscopically, all had ulcerative lesions in the stomach caused by this parasite. Histopathology showed an inflammatory infiltrate with a predominance of lymphocytes. Morphological characteristics of nematodes include the presence of three diamond-shaped lips wider than they are long, interlabia pyramidal, excretory pore located above the nerve ring, present intestinal cecum, ventriculus with five ventricular appendages, and long, winged spicules. These morphological characters, added to the number and distribution of the pre- and postcloacal papillae of the male specimens, allowed the identification of these parasites as *B. baylisi*. Scanning electron microscopy of these nematodes showed the presence of a dentigerous ridge on the inner surface of the lips in both sexes, while in males, the presence of a horseshoe-shaped median papilla was observed on the upper lip of the cloaca. Our research, therefore, adds these characteristics to the original description of *B. baylisi*, in addition to expanding the biogeographical distribution of this parasite.

1. Introduction

One of the most numerous and widely distributed predators in the Neotropics is the jacaretinga, *Caiman crocodilus* (Linnaeus, 1758), occurring in Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, Suriname, Trinidad and Tobago, and Venezuela (Farias et al., 2013; Velasco and Bulaguesa-Reina, 2016). It is an extremely adaptable species that can be found in river and lake habitats within its geographic distribution, using any body of fresh or brackish water available (Santos et al., 1996; Farias et al., 2013). These animals consume prey such as fish and mollusks, and when juvenile, they consume mainly terrestrial invertebrates (Magnusson et al., 1987; Da Silveira and Magnusson, 1999; De Matos Ramos et al., 2017).

According to Tellez (2013) and Zhao et al. (2016), several species of nematodes belonging to the families Ascarididae, Anisakidae, and Trichinellidae were reported in *C. crocodilus*. The main site of infection for these parasites is the stomach and intestine, and a high parasite load of these helminths can cause ulcers (Anderson, 2000; Mazzinghy et al., 2019). Particularly, the genus *Brevimulticaecum*, belonging to the Heterocheilidae family, causes lesions in the organs of the gastrointestinal tract in different hosts, including amphibians, reptiles, and fish (Cardoso

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et al., 2012; Muzzinghy et al., 2019).

Brevimulticaecum is one of the main nematodes that infect crocodilian reptiles that act as definitive hosts, with the infective larvae of this genus being found in amphibians, snakes, and freshwater fish (Goldberg et al., 1991, 2009; Cardoso et al., 2012). The genus *Brevimulticaecum* is characterized by having a mouth with three diamond-shaped lips, absence of dentigerous ridges, distal margins of the lips separated from the cephalic region by a distinct groove, three well-developed interlabia and presence ventriculus with short ventricular appendages (Sprent, 1979; Vicente et al., 1993; Gibbons, 2010).

The genus *Brevimulticaecum* has been little studied due to the difficulty of collecting nematodes in the crocodilians and analysis of scarce specimens (Baylis, 1923; Sprent, 1979; Vicente et al., 1993). Therefore, the aim of this study was to redescribe the nematode *Brevimulticaecum baylisi*, a stomach parasite of *Caiman crocodilus*, from subsistence hunting in the Yavarí-Mirín River, Peruvian Amazon, and we provide a list the species of *Brevimulticaecum* described in reptiles in South America.

2. Material and methods

From 2010 to 2013, viscera preserved in 10% formaldehyde from five individuals of *Caiman crocodilus* were received as a donation by subsistence hunters from the local population of the Yavarí-Mirín River (04°19'53"S, 71°57'33"W), in the northeastern Peruvian Amazon, as part of a participatory conservation program that involves local hunters in implementing wildlife management. This study was approved by the Forestry and Fauna Service of Peru (Servicio Forestal y de Fauna Silvestre; Wildlife Research Ethics Committee, protocol n° 0127–2010, 0229–2011, and 0350–2012-DGFFS-DGEFFS). In the laboratory, the organs were isolated and examined under a stereomicroscope (Leica ES2) to investigate the presence of helminths. Among all hosts, 56 nematodes that were in the stomachs of the specimens were recovered.

For morphology, the nematodes were clarified in Amann Lactophenol 90%, observed under a microscope (LEICA DM 2500) with a digital capture system (LEICA ICC50 HD) and using the Leica Application Suite software version 4.4.0, being drawn under a microscope (LEICA DM 2500) with attached camera lucida, from which photomicrographs and morphological drawings were respectively obtained. For morphometric analyses, thirty nematodes (fifteen males and fifteen females) were used. Measurements are given in micrometers, unless otherwise indicated, and are presented as mean values followed by minimum and maximum values in parentheses. The taxonomic classification of nematodes was performed according to Baylis (1923), Anderson et al. (1974, 2009), Sprent (1979), Vicente et al. (1993), and Gibbons (2010). Due to preservation in formalin, it was not possible to conduct DNA sequencing of the parasites in this study.

For scanning electron microscopy (SEM), thirteen nematodes were washed in phosphate-buffered saline (pH 7.0), post-fixed in 1% Osmium Tetroxide (OSO4) for 2 h, and then submitted to dehydration in an increasing series of ethanol from 70% ethanol until 100% for 1 h in each battery of alcohol, subsequently subjected to the critical point of CO_2, mounted on metallic aluminum supports (stubs), metallized with gold+palladium, and analyzed in a scanning electron microscope (VEGA 3 LMU/TESCAN) in the Scanning Electronic Microscopy Laboratory, Instituto de Saúde e Produção Animal na Amazônia, Universidade Federal Rural da Amazônia - UFRA, State of Pará, Brazil.

A fragment of the stomach from one of the four samples containing nematodes fixed in the mucosa with ulcerations was collected and fixed in 10% formaldehyde for histological analysis, according to Tolosa et al. (2003). Sections were 5 μm and stained with hematoxylin and eosin. The ecological indices of parasitism followed Bush et al. (1997) and Bautista-Hernández et al. (2015). Voucher specimens were deposited in the Helminthological Collection of Instituto Oswaldo Cruz: CHIOC 39619 a-d females and CHIOC 39619 e-h males.

3. Results

A total of 56 nematodes were recovered from the stomach contents of four *Caiman crocodilus* infected 80% (4/5), with a mean intensity of 14, a mean abundance of 11.2, and a range of 3–43 nematodes per infected host. Adult nematode specimens were assigned to the genus *Brevimulticaecum*. Comparative morphological and morphometric characteristics of *Brevimulticaecum* spp. are shown in Table 2 and Table 3, respectively and we provide a list of endohelminths *Brevimulticaecum* spp. in reptiles in South America (Table 1)

4. Heterocheilidae Henry and Railliet, 1912

4.1. Brevimulticaecum Mozgovoy in Skryabin, Shikobalova & Mozgovoy, 1951

4.1.1. Brevimulticaecum baylisi (Travassos, 1933) Sprent, 1979

(Based on light microscopy and scanning electron microscopy: Figs. 1 to 4)

Medium-sized nematodes. Slender body, tapering slightly at both ends. Cuticle with distinct transverse striations. Anterior end with three lips wider than long. Diamond-shaped lips, with delicate winged lateral margins and the presence of conspicuous laterally directed auricles; horn-like prolongation present in the center of the anterior border of the dorsal lip. Dorsal lip with two large, laterally directed double papillae. Ventrolateral lips with a large double papilla and conspicuous amphid. Three short interlabia. Dentigerous ridges present and interlocking processes absent. Excretory pore located above the level of the nerve ring. Long, thin, muscular esophagus ending in a glandular ventriculus with two anterior and three posterior appendages. Intestinal cecum well developed, with a narrow lumen extending anteriorly for more than half the length of the esophagus. Absence of cervical or lateral wings. Button-shaped deirids located immediately posterior to the level of the nerve ring (Fig. 1 A-H).

4.1.1.1. Male. Mean body length 10.32 mm (9.0–12.62). Width at the esophagus-intestine junction 292.38 (242.85–342.85). Esophagus length 2.05 mm (1.77–2.85) (ventriculus not included), representing 20% of body length. Length of the intestinal cecum 1.44 mm (1.14–1.88), representing 70% of the total length of the esophagus. Ventricular length and width are 10 × 13.33; the anterior ventricular appendages have a length of 5.80; the posterior lateral appendages have a length of 3.75; and the posterior median appendage has a length of 6.3. Distance from anterior end to excretory pore, nerve ring and deirids 319.04 (257.14–385.71), 389 (300–457.14), and 395.55 (323.33–460), respectively. Tail length 87.42 (80–110). Ventrally curved tail with a pointed caudal end; caudal wings absent. Tail, containing five pairs of precloacal papillae arranged as follows: one pair of more distant anterior papillae, three equidistant pairs, and one pair of lateral papillae. Five pairs of postcloacal papillae are present: two pairs paracloacal, two subventral, and one pair subdorsal. Median plaque (median papilla), which is horseshoe-shaped, is clearly visible on the anterior border of the cloaca. Lateral phasmids located posterior to the subdorsal papilla. Spicules subequal, long, filiform, winged except at the tip; one is slightly longer than the other, the first measuring 6.01 mm (5.60–6.48) and the second 6.20 mm (5.62–6.74) in length, both with expansions that end before reaching the tip of the spicules. Gubernaculum well sclerotized, measuring 180.33 (168.33–198.33) in length, thin in lateral view, tapering distally, and with two projections at the distal end (Figs. 1D, F, H, 2A, H, and 3A–H).

4.1.1.2. Female (Based on gravid females). Mean body length 11.61 mm (8.17–13.94). Width at the esophagus-intestine junction 340 (242.85–414.28). Esophagus length 2.51 mm (1.85–2.98) (ventriculus not included), representing 23% of body length. Intestinal caecum

Table 1
Endohelminths *Brevimulticaecum* spp. in reptiles in South America.

Parasite species	Host	Site of infection	Locality	References
*Brevimulticaecum pintoi*Sprent, 1979	*Caiman crocodilus*	esophagus, stomach	Paraguay	Sprent, 1979
*Brevimulticaecum baylisi*Travassos, 1933	*Caiman crocodilus*	stomach	Venezuela	Sprent, 1979
	Alligator mississippiensis			
Brevimulticaecum stekhoveni (Baylis 1947)	*Melanosuchus niger*	stomach	Brazil	Sprent, 1979
Brevimulticaecum gibsoni (Sprent, 1979)	*Melanosuchus niger*	stomach	Brazil	Sprent, 1979
Brevimulticaecum baylisi (Travassos, 1933) Sprent, 1979	*Caiman crocodilus*	stomach	Brazil	Catto, 1991
Brevimulticaecum stekhoveni (Baylis 1947)	*Caiman crocodilus*	stomach	Brazil	Catto, 1991
Brevimulticaecum baylisi (Travassos, 1933) Sprent, 1979	*Caiman crocodilus* *Melanosuchus niger*	stomach	Brazil	Vicente et al., 1993
Brevimulticaecum gibsoni (Sprent, 1979)	*Melanosuchus niger*	stomach	Brazil	Vicente et al., 1993
*Brevimulticaecum pintoi*Sprent, 1979	*Caiman latirostris* *Caiman crocodilus*	esophagus, stomach	Brazil	Vicente et al., 1993
Brevimulticaecum stekhoveni (Baylis 1947) Sprent, 1979	*Caiman latirostris, Melanosuchus niger*	Uninformed	Brazil	Vicente et al., 1993
Brevimulticaecum sp.	*Melanosuchus niger*	stomach	Brazil	Cardoso et al., 2012
Brevimulticaecum baylisi (Travassos, 1933) Sprent, 1979	*Caiman crocodilus*	stomach	Brazil	Mazzaglay, 2016
Brevimulticaecum stekhoveni (Baylis 1947)	*Caiman crocodilus*	stomach	Brazil	Mazzaglay, 2016
*Brevimulticaecum pintoi*Sprent, 1979	*Caiman crocodilus*	stomach	Brazil	Mazzaglay, 2016

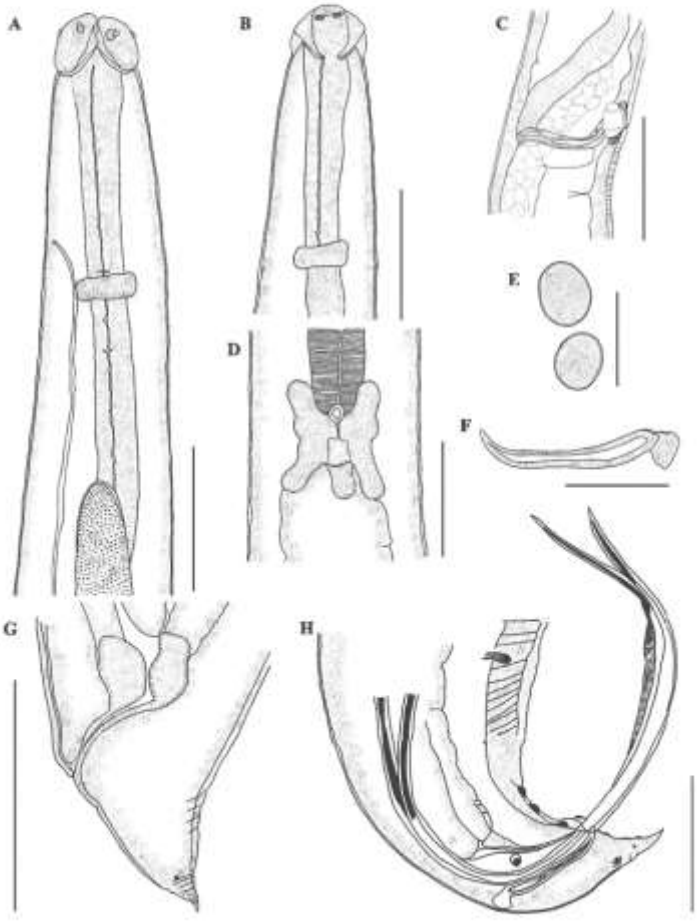

Fig. 1. *Brevimulticaecum baylisi* stomach parasite of *Caiman crocodilus* (A) Male anterior extremity, lateral view, lips, nerve ring, excretory pore, esophagus, and initial portion of the intestinal caecum can be observed. Bar = 20 µm. (B) Anterior extremity, dorsal view, dorsal lip. Bar = 20 µm. (C) Female, showing an immediately pre-equatorial vulva (v). Bar = 20 µm. (D) Ventriculus. Bar = 10 µm. (E) Eggs. Bar = 10 µm. (F) Gubernaculum. Bar = 10 µm. (G) Posterior end of female, lateral view, anus, and phasmid. Bar = 30 µm. (H) Posterior extremity of male, lateral view, observed gubernaculum, papillae and spicules. Bar = 20 µm.

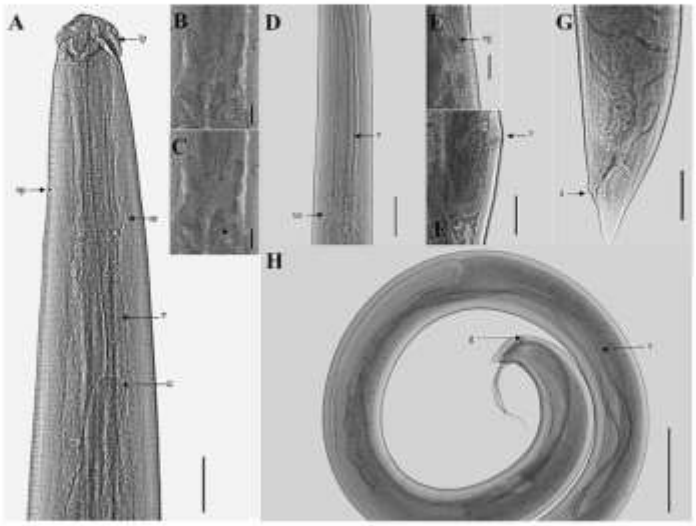

Fig. 2. *Brevimulticaecum baylisi*, stomach parasite of *Caiman crocodilus* (A) Male anterior extremity, lip (lb), nerve ring (nr), excretory pore (ep), esophagus (e), and intestinal caecum (ic). Bar = 100 µm. (B—C) Ventriculus and its appendages. Bar = 50 µm. (D) Anterior extremity, dorsal view of the esophagus (e) and ventriculus (ve). Bar = 200 µm. (E) Eggs. Bar = 100 µm. (F) Female, showing an immediately pre-equatorial vulva (v). Bar = 40 µm. (G) Female posterior end, side view, anus (a). Bar = 100 µm. (H) Posterior extremity of male, lateral view, observed gubernaculum (g) and spicules (es). Bar = 200 µm.

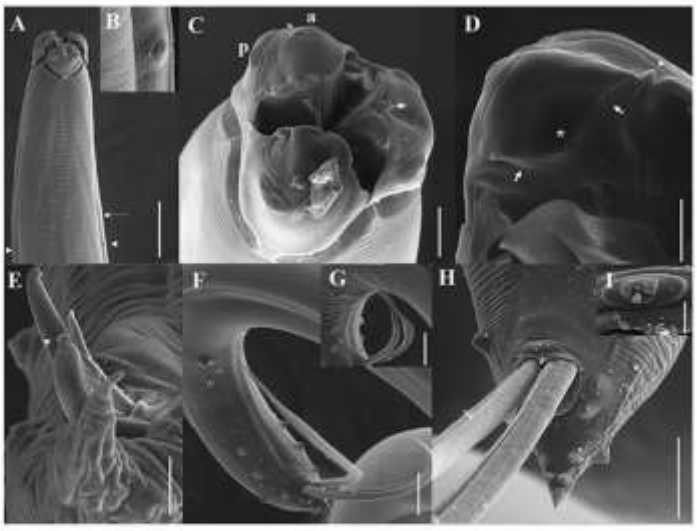

Fig. 3. Scanning electron microscopy of male *Brevimulticaecum baylisi*, stomach parasite of *Caiman crocodilus*. (A) Anterior end, showing deirids (arrowhead) and lateral line (arrow). Bar = 100 µm; (B) Insert, from the bud-shaped deirid. Bar = 5 µm; (C) Anterior extremity, latero-apical view, ventrolateral lip with amphid (a) and papilla (p); dorsal lip with a horn-shaped extension at the apex (arrow). Bar = 20 µm; (D) Ventrolateral lip, showing small dentigers (arrows), auricle (arrowhead), and cleft (*). bar = 10 µm; (E) Ventrodorsal view of the male's tail with the tip of the spicule and gubernaculum partially exposed (arrowhead). Bar = 20 µm; (F) Ventral view of the posterior end, one can observe the distribution of the papillae and the exposure of the spicules. Bar = 100 µm. (G) Posterior end, ventrolateral view of the spicules, with detail of the spicular wing. Bar = 100 µm. (H) Posterior end demonstrating postcloacal papillae. Bar = 50 µm; (I) Horseshoe-shaped median papilla. Bar = 5 µm.

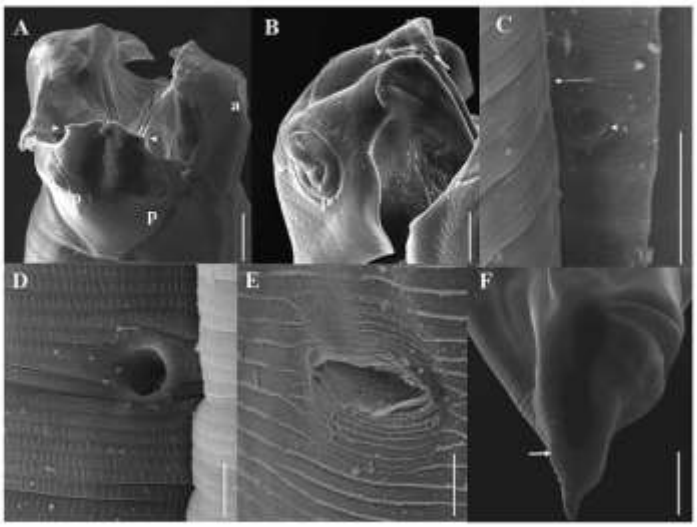

Fig. 4. Scanning electron microscopy of female *Brevimulticaecum baylisi*, stomach parasite of *Caiman crocodilus*. (A) At the anterior end, we can observe the dorsal lip with two large papillae (p) and laterally directed auricles (arrowheads), ventrolateral lip with amphid (a). Bar = 20 µm. (B) Lateral view of the dorsal lip, winged, showing the large papilla (p), dentigerous crest (arrow), and lip apex with horn-shaped expansion (arrowhead). Bar = 10 µm. (C) Lateral view, demonstrating deirid (arrowhead) and lateral line (arrow). Bar = 20 µm. (D) Excretory pore. Bar = 5 µm. (E) Vulvar region. Bar = 20 µm. (F) Conical tail, with the presence of a lateral phasmid (arrow). Bar = 50 µm.

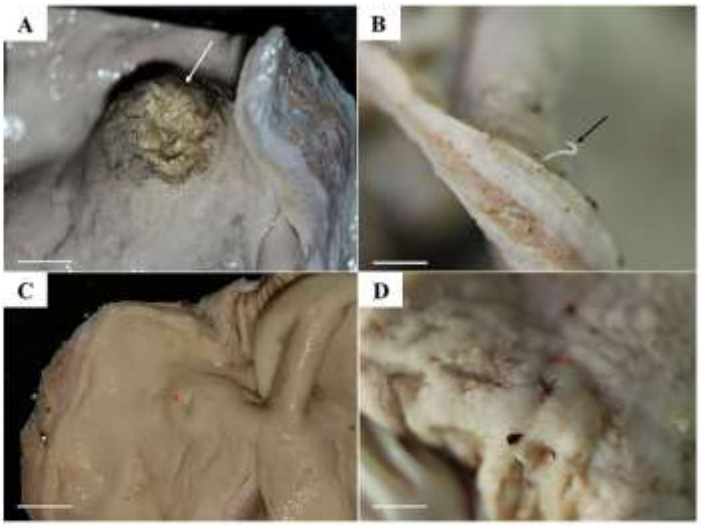

Fig. 5. Stomach of *Caiman crocodilus* parasitized by *Brevimulticaecum baylisi*: (A-B) Macroscopic view of stomach with severe ulceration and presence of helminth (arrow white and arrow black). Bar: 2 cm. (C—D) Macroscopic view of the stomach with small ulcers (arrowhead). Bar: 2 cm.

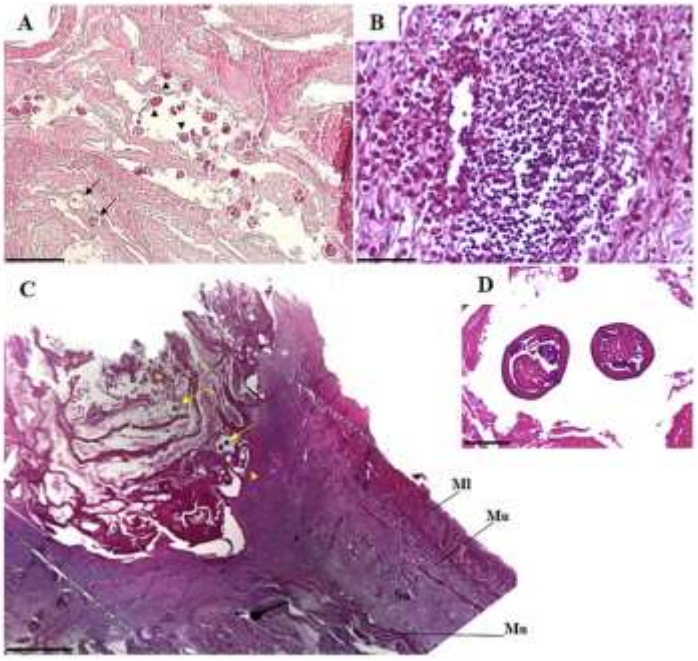

Fig. 6. Photomicrographs of the stomach of *Caiman crocodilus* parasitized by *Brevimulticaecum baylisi* stained with hematoxylin and eosin: (A) Pseudocysts are identified (arrows) and the presence of eggs (arrowhead). Bar: 200 μm. Staining: Hematoxylin and eosin. (B) In another field of observation, there is a segment of the mucosa with an intense inflammatory reaction, with the presence of polymorphonuclear cells, lymphocytes, macrophages and fibroplasia. Bar: 50 μm. (C) A small area with intact tissue, mucosa (Ma), Muscular mucosa (Mu), submucosa (Su) is observed. In the lesion, mucosal muscle is absent or fragmented (white asterisk), site of the parasitic infection with alteration of the musculature and serosa, fibrosis occurring in the area and extending to the submucosa - organized fibroplasia (arrowhead). In the submucosa and internal muscle there is a disseminated process, fibroplasia (arrowhead) and, more internally, liquefaction due to the presence of pyogenic collagen material (yellow asterisk). Some helminthic forms are identified (arrow). Bar: 2 mm. (D) Cross-section of the stomach with observation of the helminth (asterisk). Bar: 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

length 1.82 mm (1.31–2.17), representing 57% of esophagus length. Ventricular length and width 104 × 130.10; anterior ventricular appendages 5.83 length; posterior lateral appendages 3.83 length; and the posterior median appendage 7.5 length. Distance from anterior end to excretory pore, nerve ring, and deirids 349.52 (300–457.14), 432.08 (371.42–514.28), and 425.83 (346.66–520), respectively. Tail short, conical, slightly curved dorsally, measuring 225 (178.33–283.33) length. Conspicuous lateral phasmids. Distance from anterior end to vulva 5.75 mm (3.88–7.05), vulva located 49.5% from anterior end. Posteriorly directed muscular vagina. Two uteri, amphidelic. Morula stage eggs with thick shells, ovoid to round, 50 eggs measured: 72 (62.5–80) × 46 (42–52) (Figs. 2C, E, G, 4A–F).

Upon gross examination, nematodes were observed within gastric ulcers in four *C. crocodilus* specimens (Fig. 5). In histopathological analysis, we observed an area of ulceration caused by *Brevimulticaecum*. Numerous pseudocysts and several helminthic forms were identified, including eggs (Fig. 6A). It was possible to observe absent or fragmented mucosal muscle, ulcerations resulting from the parasitic infection, alterations in the musculature and serosa, and fibrosis present in the area extending to the submucosa (organized fibroplasia). In another field, it was possible to observe a segment of the mucosa with an inflammatory reaction, with the presence of neutrophils, eosinophils, lymphocytes, and macrophages, predominantly lymphocytes (Fig. 6B). In the submucosa and internal muscle, there was fibroplasia and internal liquefaction due to necrosis caused by severe tissue damage associated with parasites (Fig. 6C).

5. Discussion

This genus, *Brevimulticaecum*, is found in the stomach of *Crocodylus* spp. in Africa, India, and Australia (Vicente et al., 1993), and in the esophagus and stomach of alligators, caimans, and crocodiles in the Americas (Gibbons, 2010). The nematodes of this study found inserted in the stomach mucosa of *Caiman crocodilus* from Peru presented morphological characters compatible with the descriptions present in the literature for the genus *Brevimulticaecum*, while the main morphological characteristics that allowed the exclusion of other congener species recorded in reptiles and fish (Table 2) were the position of the excretory pore, absence of lateral line, disposition of pre- and post-cloacal papillae in males, absence of median papilla, and in females the presence of didelphic uterus (prodelphic or opstodelphic).

In our research, we identified *B. baylisi*, a species that has winged lips in the shape of a diamond, horn-like prolongation present in the center of the anterior border of the dorsal lip, and pyramidal interlabia. Presence of a lateral line, an excretory pore anterior to the nerve ring, and a ventriculus with two anterior and three posterior appendages. However, contrary to what was described by Sprent (1979), in our specimens, the presence of a dentigerous crest was observed on the internal surface of the lips, while in males, the presence of a median precloacal papilla (in the form of a horseshoe) was observed in LM and SEM.

These characters, not previously described, can be explained by the fact that there are few specimens available for analysis using SEM in the studies by Sprent (1979). Our research thus adds these characteristics to the original description. The absence of dentigerous ridges was recorded by Baylis (1923) and Travassos (1933) in the species *Brevimulticaecum baylisi*, *B. stekhoveni*, *B. tenuicolle*, and *B. pintoi*. In *B. stekhoveni*, there is a butterfly-shaped ventriculus with four appendages, as recorded in *Melanosuchus niger* in Brazil. *B. tenuicolle* recorded in the stomach of *A. mississippiensis* in the United States has a ventriculus with four short appendages and non-alate spicules. *B. pintoi*, found in the esophagus and stomach of *Caiman latirostris* (Daudin, 1801) in the Netherlands and Brazil, has spicule with a more distinct alae at the anterior end.

B. vandenbrandeni (Baylis, 1929), described in *Caiman niloticus* and *C. cataphractus* in the Congo, has elongated lips and winged margins

Table 2
Morphological characters of *Brevimulticaecum* spp. in reptiles and fish.

Morphometric characterization	*Brevimulticaecum baylisi*	*B. vandenbrandeni*	*B. baylisi*	*B. stekhoveni*	*B. pintoi*	*B. gibsoni*
Host	*Caiman crocodilus*	*Crocodylus niloticus*	*Caiman crocodilus*	*Caiman crocodilus*	*Caiman crocodilus*	*Melanosuchus niger*
Locality	Peru	Congo	Venezuela	Brazil	Amsterdam Zoo	Vienna Museum
Reference	Present study	Sprent, 1978			Sprent, 1978	
Male						
Dentigerous ridges	Present	Absent	Absent	Absent	Absent	Absent
Lips	Alate	Alate	Alate	Alate	Alate	Alate
Lips bord	Horn-like	Uniformed	Horn-like	Conspicuous	Horn-like	Absent
Interlabs	Pyramidal	Striated	Pyramidal	Sharp	Triangular	Large
Lateral alae	Present	Uniformed	Uniformed	Uniformed	Present	Present
Cervical alae	Absent	Uniformed	Absent	Absent	Absent	Absent
Excretor pore	Previous NR	Previous NR	Previous NR	At level NR	Previous NR	Posterior AN
Ventricles						
Anterior	2	2	2	2	2	2
Posterior	3	0	3	2	3	3
Papillae						
Pre-cloacais subventrais	4	4	4	4	4	Unknown
Adcloacais	1	1	1 or more	1 or 2	1 or more	Unknown
Paracloacais subventrais	2	2	2	2	2	Unknown
Papila medians ou placa mediana	1	Absent	Uniformed	Present	Uniformed	Unknown
Pos-cloacais subventrais	2	2	2	2	2	Unknown
Pos-cloacais subdorsal	2	1	1	1	1	Unknown
Spicule	Alate	Alate	Alate	Alate	Distinct alae	Unknown
Gubernaculum	Present	Present	Present	Present	Present	Unknown
Female						
Vagina	Long	Short	Long	Short	Long	Short
	Sinous	No sinous	Sinous	No sinous	No sinous	No sinous
Utero	Amphidelphic	Prodelphic	Amphidelphic	Opistodelphic	Amphidelphic	Opistodelphic

Morphometric characterization	*Brevimulticaecum baylisi*	*B. tenuicolle*	*B. regoi*	*B. alcoopagi*	*B. australensis*
Host	*Caiman crocodilus*	*Alligator mississippiensis*	*Paleosuchus matero*	Crocodiles	*Crocodilus porosus*
Locality	Perú	Germany Zoo	Paraguay River	Australia	Philippines
Reference	Present study	Sprent, 1979	Sprent, 1990		Machida, 1992
Male					
Dentigerous ridges	Present	Absent	Absent	Absent	Uniformed
Lips	Alate	No alate	Alate	Alate	Uniformed
Lips bord	Horn-like	Smooth, Horn-like	Uniformed	Uniformed	Uniformed
Interlabs	Triangular	Triangular	Triangular	Present	Uniformed
Lateral alae	Present	Uniformed	Absent	Absent	Uniformed
Cervical alae	Absent	Absent	Absent	Absent	Uniformed
Excretor pore	Previous NR	At level NR	Not seen	At level NR	Previous NR
Ventricles					
Anterior	2	2	2	2	Uniformed
Posterior	3	2	2 or 3 (digitiform bulbous)	2	Uniformed
Papillae					
Pre-cloacais subventrais	4	4	4	4	4
Adcloacais laterais	1	1	2	1	1
Paracloacais subventrais	2	2	Double	2	2
Median precloacal papilla	1	Uniformed	Absent	Presente	Double
Pos-cloacais subventrais	2	2	2	2	2
Pós-cloacais subdorsal	2	1	1	1	1
Spicule	Alate	Alate	Slender	Alate	Alate[a]
Gubernaculum	Present	Present	Present	Present	Present
Tail	Sharp point	Sharp point	Sharp point	Sharp point	Sharp point
Female					
Vagina	Long	Short	Long	Short	Uniformed
	Sinous	Thick	Sinuous	Sinuous	Uniformed
Utero	Amphidelphic	Amphidelphic	Amphidelphic	Amphidelphic	Uniformed

NR: nerve ring; a: based on the description drawing.

(Sprent, 1978). Female specimens of *Brevimulticaecum* found in the stomach of *Melanosuchus niger* from Brazil and described by Sprent (1979) as a new species due to their more anterior position of the vulva and relatively short and thick vagina were named *B. gibsoni*. Our specimens do not fit into any of the mentioned species (see Tables 2 and 3). The images illustrated by Sprent (1992) serve as a guide for identifying the genus *Brevimulticaecum* mentioned in this study.

Histological sections of the stomach infected by *B. baylisi* revealed intense inflammatory infiltrates in areas where this nematode was attached. The fixation suggests that these parasites in the gastric mucosa promote deep ulcerative lesions, similar to those observed by Cardoso et al. (2012) in *Melanosuchus niger* by *Brevimulticaecum* sp. and by *Sulcascaris sulcata* in *Caretta caretta* (Santoro et al., 2019). We place this information as preliminary data that will allow us to characterize a parasite-host relationship with a larger number of infected tissue samples in future studies. In this research, we record a redescription, with

Table 3
Morphometric comparison of *Brevimulticaecum baylisi* collected from *Caiman crocodilus* in Peru with *Brevimulticaecum* species from other crocodilians and fish hosts.

Morphometric characterization	*Brevimulticaecum baylisi*		*B. vandenbrandeni*		*B. baylisi*	*B. baylisi*		*B. stekhoveni*		*B. pintoi*		*B. pintoi*		*B. gibsoni*
Specimen sex	Male	Female	Male	Female	Male	Male	Female	Male	Female	Male	Female	Male	Female	Female
Host	*Caiman crocodilus*		*Crocodylus niloticus*		*Caiman crocodilus*	*Melanosuchus niger*		*Caiman crocodilus*		*Caiman crocodilus*		*Caiman latirostris*		*Melanosuchus niger*
Locality	Peru		Congo[a]		Venezuela	Brazil		Brazil		Amsterdam Zoo		Brazil Instituto Oswaldo Cruz		Vienna Museum
Reference	Present study		Spratt, 1970		Spratt, 1979									
Total body (L) [a]	10.34	11.61	19.7	27	11.9	10.3	8.4	21.7	30.8	13.4	14.6	10.8	8.5	12.6
Maximum body (W)[b]	292[d]	340[d]	510	800	280[d]	190[d]	200[d]	400[d]	480[d]	270	340	290	200	340
Nerve-ring (L) [b, c]	389	442	660	650	460	420	350	480	470	440	430	400	290	330
Excretory pore (L) [b, c]	319	349.5	540	540	420	360	310	410	380	400	370	not seen	260	320
Deirids [b, c]	395	425.8	–	–	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [c]	2.05	2.51	4.10	5.2	2.30	2.00	1.80	3.70	4.60	1.80	2.30	1.80	1.50	1.80
Caecum (L) [a]	1.44	1.82	2.70	3.90	1.60	1.40	1.20	2.30	3.40	1.10	1.50	1,10	1.00	1.10
Tail (L) [b]	87	225	170	340	80	120	240	190	510	70	180	60	120	250
Left spicule (L) [a]	6.01	–	1.02	–	5.10	5.20	–	0.91	–	1.80	–	1.10	–	–
Right spicule (L) [a]	6.20	–	–	–	5.10	5.20	–	0.91	–	1.80	–	1.10	–	–
Gubernaculum (L) [b]	180	–	160	–	110	190	–	150	–	180	–	140	–	–
Vulva (L) [b, c]	–	5.75	–	10.90	–	–	4.1	–	13.1	–	6.90	–	4.3	2.6
Esophagus/TL (%)	19.82	21.62	19–25	19.25	19.33	19.41	21.43	17.05	14.93	13.43	15.75	16.66	17.65	14.28
Caecum/ Esophagus L (%)	70.24	57.37	–	–	69.56	60.87	66.66	62.16	73.91	61.11	65.21	65.21	66.66	61.11
Tail/TL (%)	0.84	1.93	–	–	6.72	1.16	2.86	0.87	1.65	5.22	1.23	5.55	1.41	1.98
Vulva/TL (%)	–	49.52	–	–	–	–	48,80	–	42.53	–	47.26	–	50.58	20.63
# Specimen	15	15	2	2	–	–	–	–	–	–	–	–	–	–

Morphometric characterization	*Brevimulticaecum baylisi*		*B. tenuicolle*		*B. tenuicolle*		*B. regoi*		*B. scleropagi*		*B. australensis*	
Specimen sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Host	*Caiman crocodilus*		*Alligator mississippiensis*		*Alligator mississippiensis*		*Petenatrygon motoro*		Crocodilus Australis		*Crocodilus porosus*	
Locality	Perú		Germany Zoo		Germany Zoo		Paraguay River				Philippines	
Reference	Present study		Spratt, 1979				Spratt, 1980				Machida, 1992	
Total body (L) [a]	10.34	11.61	14.8	9.2	12.2	15.1	23.0	34.6	10.3–17.7	8.4–6.9	14.9–25	27.7–32.4
Maximum body (W)[b]	292[d]	340[d]	150[d]	180	260[d]	340[d]	550	1.100	290–520	150–690	300–730	750–950
Nerve-ring (L) [b, c]	389	442	390	350	450	520	650	750	360–590	320–670	410–670	530–670
Excretory pore (L) [b, c]	319	349.5	360	350	400	450	–	–	360–630	320–730	370–560	450–550
Deirids [b, c]	395	425.8	–	–	–	–	–	–	–	–	–	–
Total esophagus (W) [c]	2.05	2.51	2.2	1.6	2.5	3.1	2.50	3.60	1.30–2.40	1.00–2.90	2.58–4.65	4.40–5.15
Caecum (L) [a]	1.44	1.82	–	1.2	1.5	2.0	1.3	–	0.55–1.5	0.46–1.8	1.72–3.60	340–470
Tail (L) [b]	87	225	90	140	80	170	110	460	130–180	180–410	200–280	320–560
Left spicule (L) [a]	6.01	–	1.23	–	1.34	–	3.8	–	0.89–1.3	–	1.19–1.78	–
Right spicule (L) [a]	6.20	–	1.23	–	1.34	–	3.8	–	0.89–1.3	–	1.19–1.78	–
Gubernaculum (L) [b]	180	–	170	–	180	–	160	–	140–170	–	190–240	–
Vulva (L) [a,c]	–	5.75	–	4.7	–	8.80	–	15.4	–	3.9–13.1	–	11.1–13.3
Esophagus/TL (%)	19.82	21.62	14.86	17.39	20.49	20.52	10.86	10.40	–	–	16–22	–
Caecum/Esophagus L (%)	70.24	57.37	–	75	60	64.51	52	–	–	–	–	–
Tail/TL (%)	0.84	1.93	0.60	1.52	0.65	1.12	0.48	1.33	–	–	–	–
Vulva/TL (%)	–	49.52	–	51.08	–	58.27	–	44	–	–	–	38–42
# Specimen	15	15	1	1	1	1	1	1	6	9	15	10

[a] Measurements in millimeters.
[b] Measurements in micrometers.
[c] Calculated from anterior extremity.
[d] Esophageal-intestinal junction.

the addition of morphological data from SEM, of the species *B. baylisi* parasite of *C. crocodilus* from the Peruvian Amazon region.

Author contributions

RLSS, ELC, DFC performed the sampling process, morphological and morphometric analyses and wrote most parts of the manuscript. PMA, EGG, WAP designed the study, organized the field work and assisted in analyzing and interpreting histopathological results.

Discipline

Taxonomy.

Ethical statement

The study entitled "Redescription of *Brevimulticaecum bayliss*i (Travassos, 1933) Sprent (1979) (Nematoda: Heterocheilidae), parasite of *Caiman crocodilus* (Crocodylia: Alligatoridae) in north-eastern Peruvian Amazon" by Santana et al., was approved by the Forestry and Fauna Service of Peru (Servicio Forestal y de Fauna Silvestre; Ethics Committee on Wildlife Research, protocol n° 0127–2010, 0229–2011 and 0350–2012 -DGFFS-DGEFFS) following ethical standards on animal use. research.

Abbreviations: L: Length, W: Width, VL: Esophagus length, VW: Esophagus width; TL: Total body length. Notes: * Musee royal de l'Afrique Centrale, Brussels; # number

Declaration of Competing Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ARTIGO 3

Título: A molecular survey of three tick-borne pathogens in dogs from Algodoal village/Maiandeua island on the northeast coast of Pará, Brazil

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A molecular survey of three tick-borne pathogens in dogs from Algodoal village/Maiandeua island on the northeast coast of Pará, Brazil

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Abstract: *Ehrlichia* spp., *Anaplasma* spp. and *Babesia* spp. are obligate intracellular parasitic microorganisms found in the blood of domestic animals. Until then, there were no reports of these hemoparasites in dogs in the northeast of the State of Pará. The aim of this study was to record cases of natural infection by *Ehrlichia* sp., *Anaplasma* sp. and *Babesia* sp. in dogs from Ilha de Algodoal/ Maiandeua, State of Pará, Brazil, through the detection of DNA from these agents. Whole blood samples were collected from 52 animals, without considering breed, sex or age. Include results for different species of animals and parasites. Molecular analysis data showed 50% co-infection for *Ehrlichia* sp. and *Anaplasma* sp. This study allowed the detection of *Ehrlichia canis* and *Anaplasma platys* in domestic dogs on Algodoal Island, an important ecological tourism site in northern Brazil.
Keywords: hemoparasites; mammals; Brazilian amazon; zoonosis; PCR

1. Introduction

Vector-Borne Diseases (VBDs) are illnesses caused by viruses, bacteria, spirochetes, rickettsia, and parasites which are transmitted between humans, or from animals to humans through blood-feeding arthropod vectors. Since clinical manifestations can vary from no visible symptoms to severe and possibly deadly conditions, VBDs play an important role in veterinary medicine because they affect both pets and economically valuable livestock, and they are a growing public health concern due to their zoonotic potential (Savi et al., 2014; Chala et al., 2021).

Ticks are the second most common agents of VBDs, as they are hematophagous arthropods distributed worldwide, which is due to their ability to adapt to various hosts, environments, and climates (Dantas-Torres et al. 2012). In recent decades, ticks and tick-borne diseases have experienced geographic range shifts, resulting in changing rates of tick exposure and the spread of tick-borne zoonoses as Lyme disease, babesiosis, ehrlichiosis, anaplasmosis, and tularemia, which have a significant impact on public health (Araújo et al., 2015; Kilpatrick et al., 2017). Although there are hundreds of tick species found around the world, not all are known to be disease vectors, and the epidemiological importance of a given species is related to its geographic distribution. Ixodes (2 species), *Dermacentor* (2), *Amblyomma* (2), and *Rhipicephalus* (1) are the most common disease-transmitting ticks in the United States (Choi et al., 2016; Pace and O'Reilly, 2020). According to Zhao et al. (2021), in China, the most widely distributed tick genus is *Dermacentor* (574 counties), followed by Heamaphysalis (570), Ixodes (432), *Rhipicephalus* (431), *Hyalomma* (298), Argas (90), *Ornithodoros* (38), *Amblyomma* (37), and *Anomalohimalaya* (5). Brazilian ticks include 70 species, 47 in the family Ixodidae and 23 in the family Argasidae. The genera *Amblyomma* (32 species) and *Ornithodoros* (18) are the most representative (Dantas-Torres et al., 2019). Another vector species of great importance is *Rhipicephalus sanguineus* sensu lato, a cosmopolitan tick found throughout Brazil (Caetano 2016; Labruna and Pereira, 2001). It has a predilection for domestic dogs and is the vector of a series of pathogens that can infect dogs and humans, causing diseases such as ehrlichiosis, anaplasmosis, and babesiosis (Groves et al., 1975; Greene and Harvey 1990; Nava et al., 2017).

In the context of public health, VBDs represent a challenge, as they require the implementation of control and prevention strategies, both to protect health of animals and to prevent transmission of these diseases to humans. Regarding to animal health, identifying agents of VBDs is an essential instrument for veterinarians in deciding the appropriate measures for treatment and prevention (Pinto et al., 2018). Since there is no research data available, in this study we performed a molecular

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survey aiming to evaluate the prevalences of *Ehrlichia canis*, *Anaplasma platys*, and *Babesia vogeli* in the dog population of the Algodoal village/Maiandeua island on the northeast coast of Pará, Brazil.

2. Materials and Methods

2.1. Ethical aspects and Study area

From November 2019 to August 2021, 123 surgical castrations of dogs and cats of Algodoal village (-0.6066301, -47.5611076) in Algodoal-Maiandeua Environmental Protection Area (EPA), which is located in the municipality of Maracanã, Pará, Brazil, were performed within the scope of the Amazônia Veterinária Project - AVP. These procedures were authorized by the Ethics in the Use of Animals Committee (CEUA) nº23084.010805/2017 of the Federal Rural University of the Amazon, UFRA, approved in 2017, effective from November 13, 2017, to August 10, 2021.

The Algodoal-Maiandeua EPA (00°34'02" to 00°38'55" south latitude and 47°31'22" to 47°35'56" west longitude) has an area of about 3,100.34 hectares (7,661.1 acres), and is a conservation unit for the sustainable use of nature which protects two coastal islands, Algodoal and Maiandeua (Lisboa 2017; Ideflor-Bio 2019; Castro 2021), with beaches, dunes, mangroves, and wetlands that are home to fishing people and are popular with Brazilian and foreign tourists. The Amazonian equatorial climate is predominant, and the temperature averages between 26°C and 27°C, with a maximum reaching 34°C and a minimum of around 19°C, with a small thermal amplitude caused by the location conditions of the Municipality of Maracanã. It is in the Salgado region and benefits from strong sea winds. The highest rainfall index occurs in the first months of the year, from February to April, and the lowest is between September and October. These precipitations are around 2.000 mm/year (Governo do Estado do Pará, 2007).

Access to the island region from the state capital of Belém is via the BR-316 highway to the city of Castanhal and 120 km via the PA-136 and PA-318 highways, arriving at the port of Marudá District, municipality of Marapanim. Afterward, access to the island is done by boat, a crossing of about 40 minutes to the port of Vila de Algodoal (Ideflor, 2019; Castro 2021). There are still some areas covered by the original dryland forest in the municipality. With the intensity of deforestation, there is a predominance of secondary forests in the regeneration stage, and the floodplain vegetation is found on the banks of the Caripi and Maracanã rivers. In the semi-coastal and coastal areas, there is a predominance of mangroves (Governo do Estado do Pará, 2007).

Based on the registration carried out before the sterilization program, Algodoal village/Maiandeua island had 303 domiciled dogs in 2019. In addition to this, approximately 50 other non-domiciled dogs were sighted during the activities of the AVP team. The samples obtained for the present study were obtained in two work missions, the first in November 2019 and the second one in December 2020. During the first mission, 39 animals (32 dogs and 7 cats) were sterilized, and in the second one, 84 (63 dogs and 21 cats).

2.2. Sampling and collection of biological material

The study analyzed a total of 52 (32 and 20, from first and second missions, respectively) blood samples of dogs (*Canis lupus familiaris*) mixed breeds, which were randomly selected regardless of their clinical status. An epidemiological questionnaire was used by the animal guardians to obtain information about the dogs' breeding routines. The information included gender, presence of ectoparasites as ticks and fleas, if they have been living with other animals, the socioeconomic level (high, medium-high, medium, medium-low, and low), and the age group: young (up to 1 year old) and adults (1 to 5 years old).

Under chemical restraint, and by cephalic or jugular venipuncture, approximately 5 ml of blood of each animal were collected using tubes with the anticoagulant ethylenediaminetetraacetic acid (EDTA). These biological materials were properly transported in polystyrene boxes with recyclable ice. Then, in the laboratory, the samples were refrigerated at -20°C until processing.

2.3. Laboratory procedures

Genomic DNA of each sample was extracted from 300 µL of whole blood using a standard phenol-chloroform procedure, as described by Sambrook et al. (1989). DNA quality was checked by electrophoresis on an agarose gel, and the DNA was then quantified using the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, USA). Molecular detection of *Ehrlichia canis* and *Anaplasma platys* was performed following Rufino et al. (2013), while DNA research of *Babesia vogeli* was performed according to Moraes et al. (2014). Genomic DNA of blood samples of dogs previously identified as infected with E. *canis*, A. *platys*, and B. *vogeli* were used as positive controls, while sterile bi-distilled water was used as the negative control.

Since Rufino et al. (2013) and Moraes et al. (2014) are based on a nested and semi-nested PCR, respectively, to properly identify the pathogens species, amplicons of the second round PCR were cleaned using the ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fischer Scientific, Waltham, USA). Then, according to the manufacturer's specifications, purified PCR products were sequenced in both directions using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fischer

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Scientific, Waltham, USA) in conjunction with an ABI 3500 xL Genetic Analyzer (Thermo Fisher Scientific, Waltham, USA). BioEdit software (Hall, 2011) was used to align forward and reverse sequences.

2.4. Statistical analyses

Data were tabulated and treated statistically by simple descriptive percentages, implemented by Microsoft Excel 2016 software.

3. Resultados

During the missions in the Algodoal village/Maiandeua island many dogs were seen roaming the streets and beach without adequate sanitary care (Figures 1A and B). The presence of ectoparasites (mites, fleas, and ticks) was observed in 61.5% (32/52) dogs, with flea infestation being greater than that of ticks (Table 1). Coinfestation by tick and flea was the most common observed. As recorded at the time of sample collection, none of the animals received any medicine from the tutor or veterinarian. This fact has ensured the representativeness of the susceptibility of the studied animals to infection. All samples were collected from animals able to undergo castration surgery (Figures 1C, and D). Of the 52 (100%) animals analyzed, 42 were females (80.8%) and 10 were males (19.2%), with a margin of error of 10%.

Figure 1 – Algodoal Island (Maiandeua) is in the state of Pará, northern Brazil. A-B. Presence of dogs in the center of the island and on the beach C. Application of the questionnaire; C. Application of a questionnaire on the day of the dog and cat sterilization campaign.

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Ectoparasite	Mission 1 (n = 32)	Mission 2 (n = 20)	Overall (n = 52)
	Presence (%)	Presence (%)	Presence (%)
Tick (only)	4 (12.50)	0	4 (7.7)
Flea (only)	6 (18.75)	7 (35)	13 (25)
Mite (only)	0	0	0
Tick + Flea	7 (21.88)	0	7 (13.5)
Tick + Mite	0	2 (10)	2 (3.85)
Flea + Mite	3 (9.38)	0	3 (5.77)
Tick + Flea + Mite	2 (6.25)	1 (5)	3 (5.77)

Table 1 – Absolute and relative frequencies of ectoparasites in domestic dogs of Algodoal village/Maiandeua island, Pará, Brazil. n = sample size.

Sequencing of the second-round PCR amplicons of E. *canis* and A. *platys* yielded sequences, including primers, that were 389 and 212 bp long, respectively. Alignment of all sequences resulted in one haplotype to E. *canis* and as well as one to A. *platys*. The respective Blast search of these two haplotypes resulted in 100% nucleotide identity in comparison to sequences KC109445 of E. *canis* and KC109446 of A. *platys*, which were detected from dogs of Belém, Pará (see Rufino et al. 2013).

The prevalence of positive samples for E. *canis*, A. *platys* and B. *vogeli* are shown in table 2. Overall, E. *canis* DNA was detected in 6 (11.5%) dogs, while A. *platys* DNA was also detected in 6 (11.5%) dogs. Coinfections by E. *canis* and A. *platys* were detected in 4 (7.7%) of all dogs analyzed. Considering only dogs with PCR positive for E. *canis*, A. *platys* or both, i.e., 8 dogs (15.4%), coinfection rate for both missions was 50% (4/8). Tick-borne pathogens were not detected in 44 (84.6%) of all dogs. *Babesia* DNA was not detected in any sample of our study.

TBP	Mission 1 (n = 32)	Mission 2 (n = 20)	Overall (n = 52)
	Positive (%)	Positive (%)	Positive (%)
E. canis (only)	1 (3.13)	1 (5.00)	2 (3.85)
A. platys (only)	2 (6.25)	0	2 (3.85)
Babesia spp.	0	0	0
E. canis + *A. platys*	4 (12.5)	0	4 (7.70)
Overall	7 (21.88)	1 (5.00)	8 (15.40)

Table 2 – Absolute and relative frequencies of tick-borne pathogens (TBP) in dogs of Algodoal village/Maiandeua island, Pará, Brazil. n = sample size.

Reference	Locality (n)	Prevalence (%)
Diniz et al. (2007)[1]	Botucatu – SP (198)	77.7
Ueno et al. (2009)[1]	Botucatu – SP (70)	40,0
Macieira et al. (2005)[1]	Rio de Janeiro – RJ (226)	15.0
Dagnone et al. (2003)[1]	Londrina – PR (129)	22.0
Dagnone et al. (2009)[2]	Jaboticabal – SP (25)	88.0
Nakaghi et al. (2008)[2]	Jaboticabal – SP (30)	53.3
Faria et al. (2010)[2]	Jaboticabal – SP (40)	72.5
Bulla et al. (2004)[2]	Botucatu – SP (217)	30.9
Santos et al. (2009)[2]	Ribeirão Preto – SP (221)	38.9
Carvalho et al. (2008)[2]	Ilhéus-Itabuna – BA (153)	7.8
Dagnone et al. (2009)[2]	Campo Grande – MS (26)	38.4
Soares et al. (2017)[2]	Campo Grande – MS (181)	55.75
Ramos et al. (2009)[2]	Recife - PE (100)	57.0
Sousa (2006)[2]	Cuiabá – MT (60)	20.0
Rufino (2013)[1]	Belém – PA (200)	24.0
Costa Jr (2007)[3]	Lavras – MG (97)	1.03
Costa Jr (2007)[3]	Belo Horizonte – MG (49)	24.49
Costa Jr (2007)[3]	Nanuque – MG (102)	26.47

Table 3 – Summary of prevalence of *Ehrlichia canis* in Brazilian domestic dogs based on molecular diagnosis. 1PCR, 2nested PCR, 3Real time PCR

Reference	Locality (n)	Prevalence (%)
Lasta et al. (2013)[2]	Porto Alegre – RS (199)	14.07
Soares et al. (2017)[2]	Campo Grande – MS (181)	16.96
Melo et al. (2016)[2]	Poconé – MT (320)	7.19
Costa (2015)[1,3]	Goiânia – GO (500)	6
Vieira (2017)[3]	Espírito Santo – ES (378)	6
Costa Jr (2007)[3]	Lavras – MG (97)	7.22
Costa Jr (2007)[3]	Belo Horizonte – MG (49)	4.08
Costa Jr (2007)[3]	Nanuque – MG (102)	19.61

Table 4 – Summary of prevalence of *Anaplasma platys* in Brazilian domestic dogs based on molecular diagnosis. 1PCR, 2nested PCR, 3Real time PCR.

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4. Discussion

Over the past years, there has been a significant increase in the number of cases of VBDs in several regions worldwide (Gubler 2010; Chala and Hamde 2021), and preventive actions such as (i) identifying the circulating pathogens, (ii) implementing effective measures for epidemiological surveillance, and (iii) establishing appropriate vector management strategies are essential in reducing the incidence and mitigating the impact of these diseases.

In this context, to the best of our knowledge, this is the first survey of vector-borne pathogens carried out in Algodoal village/Maiandeua island, on the northeast coast of Pará, Brazil, which has a domestic dog population living in very close contact with local vegetation, wild animals, migratory birds, and the local human population, as well as tourists. These factors, in addition to the environmental conditions, may favor the proliferation of ticks and, consequently, the dissemination of vector-borne pathogens (Temoche et al., 2022).

Regarding the results of the present study, the overall prevalence of E. *canis* in domestic dogs of Algodoal village/Maiandeua island was lower than that observed for most records from other Brazilian localities (Table 3), including Belém, Pará, Brazil, which is 140 km from our study area. On the other hand, the A. *platys* prevalence we found here is similar to that recorded elsewhere (Table 4). In general, higher frequencies of infection are expected in regions where the tick vector is more abundant, and differences in the prevalence of vector-borne pathogens can also be explained by the methodologies used for diagnosis, geographic regions evaluated, sampling period, vector, and host (Benavides-Arias and Soler-Tovar 2020). Costa Jr. (2007) provides an example where the overall prevalence of E. *canis* and A. *platys* was found to be lower in urban areas compared to rural areas in Minas Gerais, Brazil. In that study, infestation by R. *sanguineus* ticks was identified as the sole risk factor contributing to this difference, particularly for E. *canis*. In this sense, according to our observation of a low prevalence of tick infestation, this may be the case in Algodoal village or Maiandeua island.

Despite the prevalence of E. *canis* and A. *platys* in Algodoal village/Maiandeua island, one of the most notable results of this study is the high frequency of coinfections in comparison to individual infection rates. Among factors potentially contributing to this condition, we point out the unrestricted movement of dogs within Algodoal village/Maiandeua island, which has led to close contact between animals in a territorially limited area, as well as the lack of preventive measures to control tick infestation. According to Sousa et al. (2021), anaplasmosis and ehrlichiosis are recurrent diseases, and their coinfection has been growing among domestic animals. It is necessary to implement the best way of diagnosing these diseases by associating, in addition to clinical signs, molecular tests. In our research, animals coinfected by *Ehrlichia* and *Anaplasma* did not show significant clinical signs, although in some cases, oral and gingival mucous membranes were slightly pale, capillary refill time increased (> 3 seconds), and eye discharge was observed. Clinical signs are evidenced mainly in young animals (5–10 months old) and may also be related to endoparasites.

Another relevant result of this study is our failure to detect *Babesia* spp., which may reflect our small sample size (15% of the total population, i.e., domiciliated and non-domiciliated dogs), as well as a low prevalence of infection or even a complete absence of this pathogen in that locality. Unfortunately, data on canine babesiosis in northern Brazil is scarce. Panti-May and Rodriguez-Vivas (2020), investigating available data on canine babesiosis in Latin America and the Caribbean (from January 2000 to December 2019), reported only 34 published studies, which showed prevalence varying considerably based on parasite species and geographic location, with values close to zero to 26.2%. In northern Brazil, an exception is the study of Moraes et al. (2014), who, based on the same detection protocol used in the present study, reported a prevalence rate of B. *vogeli* in domestic dogs in Belém, Pará, Brazil, equal to 15.7% (27/172), which, in comparison to other studies (see Panti-May and Rodriguez-Vivas 2020), is moderately high. However, since it was based on a relatively smaller sample size, the contrast with our findings supports the hypothesis of a low prevalence or even the absence of *Babesia* in Algodoal village or Maiandeua island. Confirming the occurrence of *Babesia* spp. in this area requires extensive research, including long-term epidemiological surveillance.

It is worth highlighting that Algodoal village/Maiandeua island, located on the northern coast of Brazil, play a role as an important stopover and wintering site for a number of aquatic migratory birds (Gonçalves et al., 2007; Krietsch et al., 2017). The ecology of aquatic birds, such as their migratory behavior, diet, habitat use, and aggregation habits, has direct effects on the global distribution and diversity of vectors and pathogens (Morshed et al., 2005; Abdelbaset et al., 2023). Thus, this reinforces Panti-May and Rodriguez-Vivas (2020), who highlight the need for further research in order to enhance our understanding of the ecology and epidemiology of *Babesia* spp. in dogs in Latin America and the Caribbean.

In general, our results contradict the hypothesis that animals with an exclusively outdoor lifestyle, living virtually in sympatry with sylvatic species, and without regular controls and treatments, as in the case of the dogs studied here, are more exposed to the risk of infection. Based on our observations, especially during the second mission, the most coherent explanation for this is the low prevalence of tick infestation.

The transmission of VBDs requires an introduced and/or established vector population, a pathogen, and suitable environmental and climatic conditions across the full cycle of VBD transmission (Randolph and Rogers 2010). It is widely known that ticks are susceptible to climatic determinants, specifically humidity and temperature. Indeed, a high incidence of tick-borne disease has been reported to be linked with moderate winters and humid, warm summers, although incidence may also be affected by the influence of climate on recreational activities (Ostfeld and Brunner 2015). Since the temperature in Algodoal village/Maiandeua island is favorable, its low prevalence of ticks, as we observed here, seems to be influenced by other biotic

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and/or abiotic factors. Showler et al. (2019) studied the relationships between some abiotic and biotic factors that influence ixodid distribution. observed (i) exposure of lone star tick, *Amblyomma* americanum, and *Rhipicephalus* microplus eggs to hypersaline water is lethal; (ii) although intermittent hypersaline flooding kills ixodid eggs, saline soil was not particularly toxic; (iii) when relative humidity is relatively low, desiccation causes high egg mortality on dry soil, regardless of salinity; and (iv) substantial year-round populations of mud flat fiddler crabs, Uca rapax (Decapoda: Ocypodidae), on saline soil eliminated 80% of an *Amblyomma* americanum egg masses overnight. Since Algodoal village/Maiandeua island are situated in the "salgado paraense" region, it is reasonable to believe that at least the influence of saltwater toxicity and predation by crabs on survival and the abundance of ticks occurring there should not be overlooked.

Although the results of our study are primarily relevant to veterinary concerns, its significance for public health should not be disregarded because the literature has reported an increasing number of human anaplasmosis cases caused by A. *platys* and human ehrlichiosis cases caused by E. *canis* in recent years (Perez et al., 2006; Maggi et al., 2013; Arraga-Alvarado et al., 2014). *Anaplasma* and *Ehrlichia* are responsible for causing nonspecific febrile illnesses that are mostly self-limiting. However, older individuals, patients with underlying medical conditions, or those with weakened immune systems face a higher risk of experiencing severe illness or even death if prompt and appropriate treatment is not administered.

5. Conclusion

E. *canis* and A. *platys* were responsible for naturally infecting domestic dogs in Algodoal/Maiandeua island, north of Brazil. These preliminary data show the need for more comprehensive work with greater sampling effort and the collection of vectors for the diagnosis of infectious agents transmitted in the APA of Algodoal.

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ARTIGO 4

Título: *Ozolaimus megatyphlon* and *Ozolaimus cirratus* parasitizing the *Iguana iguana* (Linnaeus, 1758) from Marajó Island, Pará, Brasil: new occurrence and morphological redescription

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Original Article

Ozolaimus megatyphlon and *Ozolaimus cirratus* parasitizing the *Iguana iguana* (Linnaeus, 1758) from Marajó Island, Pará, Brasil: new occurrence and morphological redescription

Ozolaimus megatyphlon e *Ozolaimus cirratus* parasitando *Iguana iguana* (Linnaeus, 1758) da Ilha de Marajó, Pará, Brasil: nova ocorrência e redescrição morfológica

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Abstract

This study aimed to redescribe two species of *Ozolaimus*, parasites of free-living green iguanas native to Marajó Island. The gastrointestinal system of four iguana specimens was evaluated for the presence of helminths. Altogether, 12,028 nematodes were found, with a prevalence of 100%, an infection range of 780 to 7,736 nematodes, an infection intensity of 3.007, and a mean abundance of 3,007. Light microscopy and scanning electron microscopy were used to determine the species of nematodes found. The cecum was the site of infection that had the highest parasitic load. Morphologically, the nematodes were compatible with the genus *Ozolaimus* Dujardin, 1844, with the species *Ozolaimus megatyphlon* (Rudolphi, 1819) Dujardin, 1845, and *Ozolaimus cirratus* Linstow, 1906. Scanning electron microscopy showed the presence of small structures (serrated in *Ozolaimus cirratus* and rounded in *Ozolaimus megatyphlon*) located below the esophageal leaves. We also evidenced the phasmids in both species; this is the first record of these structures in nematodes of the genus *Ozolaimus*. In addition, this work expands the records on the geographic distribution of these parasites.

Keywords: Green iguana, parasites, nematodes, *Ozolaimus*, Pará.

Resumo

Este estudo tem como objetivo redescrever duas espécies de *Ozolaimus*, parasitas de iguanas verdes de vida livre nativas da Ilha de Marajó. O sistema gastrointestinal de quatro espécimes de iguana foi avaliado quanto à presença de helmintos. Ao todo, foram encontrados 12.028 nematoides, com prevalência de 100%, intervalo de infecção de 780 a 7.736 nematoides, intensidade de infecção de 3.007 e abundância média de 3.007. Microscopia de luz e microscopia eletrônica de varredura foram utilizadas para determinar as espécies de nematoides encontradas. O ceco foi o local de infecção que apresentou maior carga parasitária. Morfologicamente, os nematoides eram compatíveis com o gênero *Ozolaimus* Dujardin, 1844, com as espécies *Ozolaimus megatyphlon* (Rudolphi, 1819) Dujardin, 1845 e *Ozolaimus cirratus* Linstow, 1906. A microscopia eletrônica de varredura mostrou a presença de pequenas estruturas (serrilhadas em *Ozolaimus cirratus* e arredondadas em *Ozolaimus megatyphlon*) localizado abaixo das folhas esofágicas. Também foram evidenciados os fasmídeos em ambas as espécies. Este é o primeiro registro dessas estruturas em nematoides do gênero *Ozolaimus*. Além disso, este trabalho amplia os registros sobre a distribuição geográfica desses parasitos.

Palavras-chave: Iguana verde, parasitas, nematoides, *Ozolaimus*, Pará.

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Introduction

Iguana iguana (Linnaeus, 1758), popularly known as the green iguana, belongs to the order Squamata (Lepidosauria: Reptilia), the most diversified among the group and one of the most important, which includes about 19 families and 4,500 species of lizards (Barten, 2006; Vitt & Caldwell, 2008). *Iguana iguana* is distributed throughout the Americas, including Brazil and occurs in the Amazon, Caatinga and Pantanal biomes (Campos & Desbiez, 2013).

Due to their low cost and ready availability, green iguanas have become one of the most popular unconventional pets in Brazil, making them economically important (Bauer & Bauer, 2014). Additionally, they are herbivorous, which many owners prefer, as they do not have to deal with providing insects or rodents for food (Barten, 1993).

These reptiles are hosts for a wide variety of parasites, which can be acquired by ingestion of contaminated plant material, coprophagy, geophagy, or active penetration by nematode larvae (Anderson et al., 2009). The authors Loukopoulos et al. (2007) and Breves et al. (2011) demonstrated that helminths of the Oxyurida order are commonly found parasitizing the gastrointestinal system of green iguanas.

Despite the growing number of studies related to host parasites of green iguanas in Brazil, most studies are concentrated in the Southeast, Midwest, and Northeast regions of the country, leaving gaps regarding helminth fauna in the northern region. The Amazon region still lacks information on helminths related to lizards. With that in mind, this study aimed to redescribe two species of the genus *Ozolaimus* of *Iguana iguana* from Marajó Island in the State of Pará.

Material and Methods

From 2019 to 2021, four free-living specimens of *I. iguana* were acquired dead from residents of the municipality of Soure (00° 43' 00" S; 48° 31' 24" W), Marajó Island, State of Pará. The research was carried out under authorization from Sisbio nº 68028. The organs of the digestive system, such as the esophagus, stomach, small intestine, and large intestine (colon, cecum, and rectum), were transported refrigerated to the laboratory. In the laboratory, each organ was isolated in plastic trays containing 0.9% NaCl saline and analyzed under a Leica ES2 stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany) to investigate the presence of helminths.

The collected nematodes were washed in 0.9% NaCl physiological solution, fixed in AFA solution (93 parts of 70% ethyl alcohol, 5 parts of formaldehyde, and 2 parts of glacial acetic acid), and stored in 70% alcohol. For light microscopy (LM), the nematodes were clarified in a 30% Lactophenol Aman solution and photographed in a Leica DM2500 microscope with a DFC310 FX digital capture system with Leica Application Suite V4.4 software (Leica Microsystems GmbH, Wetzlar, Germany). They were drawn and measured using a Leica DM2500 microscope with an imaging tube attached. The drawings were measured with the aid of a ruler, and the measurements were converted to micrometers or millimeters according to the Leica DM2500 (Leica Microsystems GmbH, Wetzlar, Germany) measurement conversion table. For morphometric analysis, 25 males and 20 females were used. After those procedures, the nematodes were stored in glycerin alcohol (70% ethanol with 5% glycerin). Measurements are given in millimeters, unless otherwise noted, and are presented as average values followed by minimum and maximum values in parentheses.

For scanning electron microscopy (SEM), the nematodes fixed in AFA solution were washed with distilled water, post-fixed in 1% osmium tetroxide for 2 hours, and then submitted to dehydration in an increasing series of ethanol from 70% ethanol until 100% for 1 hour in each battery of alcohol, subsequently subjected to the critical point of CO_2 model K850 Critical Point Dryer (Quorum Technologies Ltd., England), mounted on metallic aluminum supports (stubs), metallized with gold+palladium, and analyzed in a scanning electron microscope model VEGA 3 LMU (TESCAN, Brno, Czech Republic). Scientific articles and dichotomous keys were used to identify the species: Dosse (1942), Leussink (1958), Vicente et al. (1993), Anderson et al. (2009), and Gibbons (2010).

Voucher specimens for the parasites were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Manguinhos, Rio de Janeiro, Brazil, as: CHIOC 39620 a-h for males and females of *O. cirratus* and CHIOC 39621 a-h for males and females of *O. megatyphlon*, correspondingly.

Results

A total of 12,028 nematodes were recovered from four specimens of *I. iguana*, with prevalence of 100% (n=4), mean intensity of infection of 3,007, mean abundance of 3,007, and range of infection of 780 to 7,736 nematodes. The parasites were found (mixed infection) in the small and large intestine (colon, cecum, and rectum).

The cecum was the site with the highest rate of infection. The specimens collected are morphologically compatible with the genus *Ozolaimus* Dujardin, 1844. In our study, we identified the species *Ozolaimus megatyphlon* (Rudolphi, 1819), Dujardin, 1845, and *Ozolaimus cirratus*, and their morphometries were compared to others already described in the literature for these two species (Tables 1 and 2).

Order Oxyurida,

Family Pharyngodonidae Travassos, 1920

Genus *Ozolaimus* Dujardin, 1844

Specie *Ozolaimus megatyphlon* (Rudolphi, 1819) Dujardin, 1845

Ozolaimus cirratus Linstow, 1906

Table 1. Comparison of morphometric data of specimens of *Ozolaimus megatyphlon* from *Iguana iguana* on Marajó Island, PA, with data from other authors. a: The front-end, b: µm, #: number.

	Ozolaimus megatyphlon		***O. megatyphlon***		***O. megatyphlon***		***O. megatyphlon***	
Host	***Iguana iguana***		*Iguana iguana rhinolopha*		*Iguana iguana*		*Iguana iguana*	
Location	**Marajó, Pará, Brazil**		Mexico		Alagoas, Maranhão, Goiás and Mato Grosso, Brazil		Maranhão, Brazil	
Habitat	**Cecum e intestine**		Cecum		Cecum, colon and rectum		Large intestine	
References	**Present study**		Caballero, 1938		Breves et al., 2011		Otávio et al., 2018	
	Female	**Male**	Female	Male	Female	Male	Female	Male
Total body length	**4.80–7.57**	**4.71–6.28**	6.25–7.40	4.85–5.90	4.99–6.97	3.67–4.76	7.10–8.10	5.40–5.50
Body width	**0.13–0.80**	**0.33–0.61**	0.62–0.72	0.39	0.56–0.85	0.34–0.40	0.70–0.80	0.50–0.70
Nerve Ring[a]	**0.22–0.37**	**0.10–0.80**	–	–	0.30–0.80	0.20–0.30	–	–
Excretory pore	**1.30–2.74**	**1.08–2.14**	–	–	–	–	2.40–2.80	1.80–2.00
1a – Length of the 1º part of the esophagus	**0.74–1.20**	**0.50–0.86**	–	–	1.04–1.16	0.66–0.96	1.15–1.36	0.90–0.95
1b – Width of 1st part of the esophagus	**0.07–0.24**	**0.10–0.16**	–	–	–	–	–	–
2a – Length 2nd part of the esophagus	**0.42–1.37**	**0.30–1.02**	–	–	1.39–1.71	0.89–1.22	1.17–1.62	1.10–1.16
2b – Width of the 2nd portion of the esophagus	**0.05–0.19**	**0.04–0.53**	–	–	–	–	–	–
Basal bulb length	**0.17–0.36**	**0.14–0.28**	–	–	0.18–0.25	0.10–0.19	0.21–0.23	0.22
Basal bulb width	**0.13–0.29**	**0.14–0.22**	–	–	0.18–0.28	0.16–0.20	0.25–0.28	0.20–0.23
Total esophagus	**1.56–2.77**	**1.20–1.90**	–	–	2.43–2.87	1.68–2.09	2.45–2.90	2.00–2.10
Spicule	–	**0.80–2.10**	–	1.07–1.18	–	0.72–1.03	–	1.01–1.20
Tail[b]	**200–510**	**70–200**	–	–	–	–	–	–
Distance from vulva to anus	**1.00–1.94**	–	–	–	–	–	1.57–1.80	–
Distance from vulva to end of tail	**1.03–2.50**	–	1.35–2.65	–	0.98–1.58	–	–	–
Length of eggs[b]	**100–980**	–	130–140	–	100–150	–	100–120	–
Egg width[b]	**50–80**	–	69–70	–	60–90	–	60–70	–
# specimes	**20**	**25**	–	–	–	–	10	10

Table 2. Comparison of morphometric data of specimens of *Ozolaimus cirratus* from *Iguana iguana* from Marajó Island, with data from other authors. a: The front-end, b: µm, #: number.

	Ozolaimus cirratus		***O. cirratus***		***O. cirratus***		***O. cirratus***		***O. cirratus***	
Host	***Iguana iguana***		*Iguana tuberculata*		*Iguana tuberculata*		*Iguana iguana*		*Iguana iguana*	
Location	**Marajó, Pará, Brazil**		Alemanha		México		Alagoas, Maranhão, Goiás and Mato Grosso, Brazil		Maranhão, Brazil	
Habitat	**Large intestine, Ceca**		Intestine		?		cecum and colon		Small intestine	
References	**Present study**		Linstow, 1906		Dosse, 1942		Breves et al., 2011		Otávio et al., 2018	
	Female	**Male**	Female	Male	Female	Male	Female	Male	Female	Male
Total body length	**4.46–7.77**	**5.00–7.28**	6.20	5.66	8.26–10.95	5.17–6.52	6.38–8.73	4.90–6.54	7.70–8.00	5.75–6.90
Body width	**0.53–0.86**	**0.40–0.63**	0.79	0.53	0.75–1.00	0.50–0.58	0.84–1.10	0.57–0.60	0.80–1.00	0.60–0.67
Nerve Ring[a]	**0.29–0.37**	**0.17–0.29**	–	–	0.20–0.31	0.25–0.38	0.40–0.60	0.20–0.30	–	–
Excretory pore	**1.66–3.14**	**1.19–2.01**	–	–	2.00–3.57	1.50–2.29	–	–	2.60–2.80	2.32–2.50
1a – Length of the 1st part of the esophagus	**0.71–1.22**	**0.52–0.74**	–	–	0.78–1.06	0.58–0.83	1.05–1.16	0.74–0.91	1.10–1.15	0.81–0.91
1b – Width of 1st part of the esophagus	**0.13–0.31**	**0.14–0.20**	–	–	–	–	–	–	–	–
2a – Length 2nd part of the esophagus	**0.50–1.00**	**0.36–0.72**	–	–	0.90–1.33	0.73–1.00	1.19–1.36	0.89–1.13	1.05–1.17	0.77–1.02
2b – Width of the 2nd portion of the esophagus	**0.04–0.17**	**0.06–0.13**	–	–	–	–	–	–	–	–
Basal bulb length	**0.17–0.38**	**0.14–0.27**	–	–	0.11–0.23	0.15–0.20	0.29–0.37	0.20–0.27	0.30–0.35	0.18–0.28
Basal bulb width	**0.17–0.26**	**0.11–0.20**	–	–	–	–	0.27–0.34	0.21–0.25	0.25–0.30	0.24–0.27
Total esophagus	**1.55–2.80**	**1.12–2.15**	–	–	–	–	2.25–2.45	1.65–2.04	2.20–2.30	1.70–1.92
Spicule	–	**1.50–2.37**	–	2.20	–	1.80–2.10	–	2.08–2.37	–	1.80–2.50
Tail[b]	**170–510**	**110–230**	–	–	300–480	110–180	–	–	–	–
Distance from vulva to anus	**1.05–1.80**	–	–	–	–	–	–	–	1.98–2.80	–
Distance from vulva to end of tail	**1.42–2.22**	–	–	–	2.00–3.47	–	1.40–3.03	–	–	
Length of eggs[a]	**80–120**	–	90	–	140–150	–	120–140	–	110–130	–
Egg width[a]	**50–70**	–	60	–	60–70	–	60–100	–	60	–
# specimes	**20**	**25**	–	–	20	10	–	–	10	10

Ozolaimus megatyphlon (based on light microscopy and scanning electron microscopy. Figures 1 to 4; Table 1)

Medium-sized parasite, rounded body with cuticle completely striated transversely. Two lateral lips and a triangular oral opening, with small, rounded projections below the esophageal leaves. Esophagus long, thin, almost cylindrical, and divided into two portions: the first comprises the dilatation region, and the second goes to the bulb, the second portion being longer than the first. Bulb well developed; excretory pore immediately anterior or at the level of the bulb; nerve ring near the first portion of the esophagus. Males have a projection of six cuticular membranes from the esophageal segment. Spicule short and pointed. Genital cone present. Tail differentiated, short, and curved, containing a pair of precloacal papillae, a pair of postcloacal papillae, and a pair of caudal papillae. Females with vulva covered by a very prominent vulvar lip and a long uterus. Gravid females have ovoid, thin-shelled eggs and no embryonated.

Based on 25 male specimens: body 5.31 mm (4.71–6.28) length and 0.42 (0.33–0.61) wide at the bulb region. Distances from anterior end to nerve ring and excretory pore 0.26 (0.10–0.80) and 1.74 (1.08–2.14), respectively.

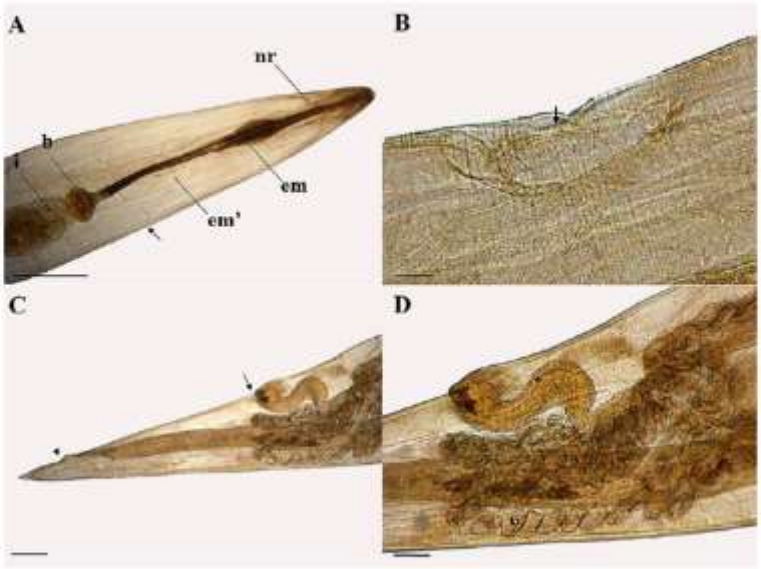

Figure 1. Light microscopy of female *Ozolaimus megatyphlon*, a parasite of *Iguana iguana*. A. Anterior extremity, nervous ring (nr), esophagus first portion (em), and muscular esophagus second portion (em'), prebulbar excretory pore (arrow), bulb (b), intestine (i). Bar=300µm. B. Excretory pore (arrow). Bar=60µm. C. Posterior end, lateral view, the vulva (arrow) and anal opening (arrowhead). Bar=300µm. D. Muscular vagina (*), uterus with eggs (e). Bar=100µm.

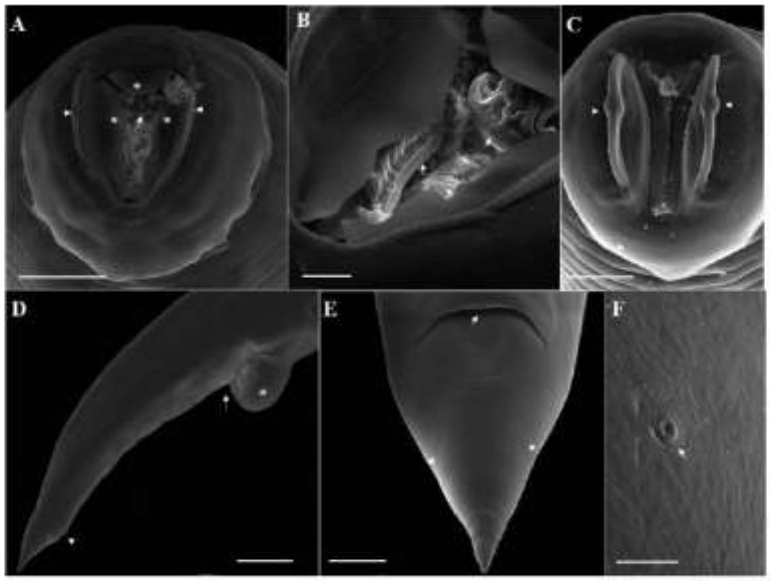

Figure 2. Scanning electron microscopy of female *Ozolaimus megatyphlon*, a parasite of *Iguana iguana*. A. Anterior end, projection of 3 tripartite cuticular membranes from the esophageal segment (*). Orifice of the amphids canal (arrowhead) and esophageal leaves (arrow). Bar=50µm. B. Esophageal leaves with rounded structure (arrow). Bar= 10µm. C. Anterior extremity, slit mouth, and presence of lips with lateral cephalic papilla (arrowhead). Bar=20µm. D. Posterior end, lateral view, vulva (arrow) whose anterior lip is projected (*), and anal opening (arrowhead). Bar=200µm. E. Posterior end, ventral view, tail, anal opening (arrow), and phasmids (arrowhead). Bar=50µm. F. Phasmid (arrow). Bar=5µm.

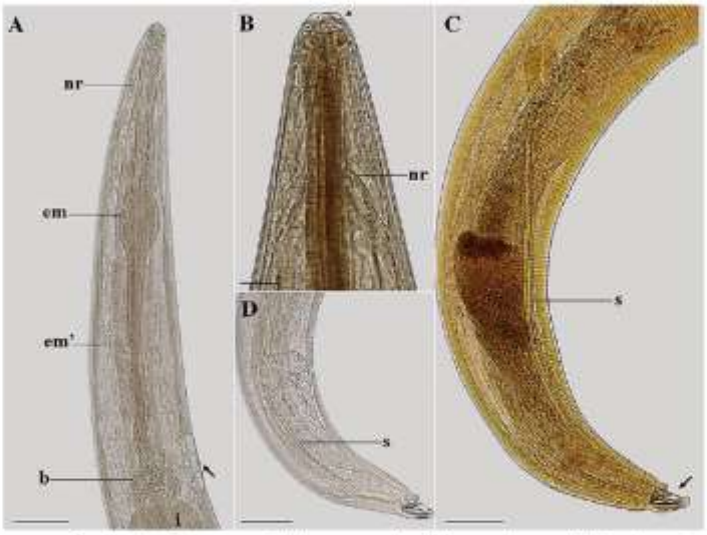

Figure 3. Light microscopy of male *Ozolaimus megatyphlon*, a parasite of *Iguana iguana*. A. Anterior extremity, nerve ring (nr), esophagus first portion (em), and muscular esophagus second portion (em'), prebulbar excretory pore (arrow), bulb (b) and intestine (i). Bar=100µm. B. Anterior extremity, lips (arrowhead) and nerve ring (nr). Bar=50µm. C-D. Posterior end, lateral view, the spicule (s) and tail (arrow). Bar=200µm.

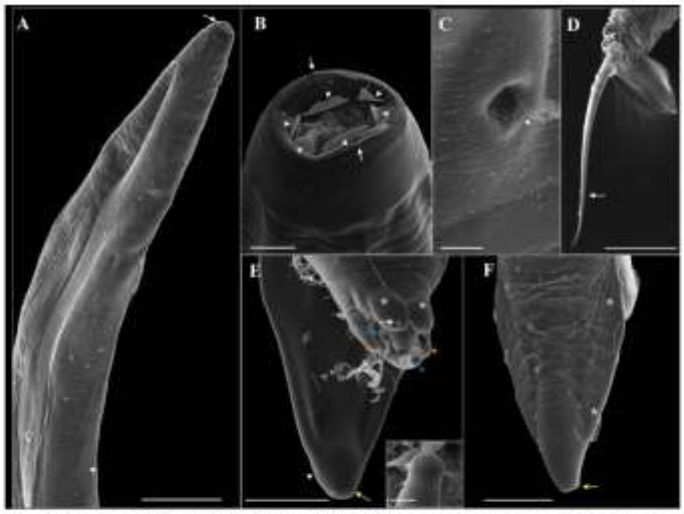

Figure 4. Scanning electron microscopy of male *Ozolaimus megatyphlon*, a parasite of *Iguana iguana*. A. Anterior extremity, observe the cephalic region (arrow) and excretory pore (arrowhead). Bar=500µm. B. Anterior extremity in the cephalic region, one can observe the mouth in a vertical slit delimited by two conspicuous and hemispherical lateral lips containing amphid each (arrow), projection of six cuticular membranes (arrowhead). Bar=20µm. C. Observe the excretory pore (arrowhead). Bar=5µm. D. Posterior end, exposed spicule (arrow) and retracted tail. Bar=100µm. E. Posterior end, lateral and ventral view, two pairs of papillae can be seen: a large pair (*), in a ventral and precloacal position and the other small pair located at the tip of the tail (arrow). Around the cloaca there are two pairs of appendages: a large pair a in the dorsolateral position (orange arrowhead), bar= 50µm, and the other small membranous pair occupying a ventrolateral position, (white arrow) and genital cone (blue arrowhead). Caudal papillae (yellow arrow). Phasmids (white arrowhead). Bar=50µm; Insert genital cone (blue arrowhead). Bar=10µm. F. Caudal wings (*) well developed are present, originating immediately anterior to the tail insertion and ending immediately anterior to the caudal papillae (yellow arrow). Bar=25µm.

Elongated esophagus measuring 1.63 (1.20–1.90) in length, divided into two portions where the first portion measures 0.72 (0.50–0.86) in length and 0.14 (0.10–0.16) wide; the second portion measures 0.69 (0.30–1.02) in length and 0.09 (0.04–0.53) wide. Esophageal bulb measuring 0.19 (0.14–0.28) in length and 0.18 (0.14–0.22) in width. A differentiated shape of the end of the spicule: spicule is short and pointed. Spicule 1.28 (0.80–2.10) in length. Tail measuring 140 (70–230) µm in length. Phasmids present.

Based on 20 female specimens: body 6.89 mm (4.80–7.57) length and 0.64 (0.13–0.80) wide at bulb region. Distances from anterior end to nerve ring and excretory pore 0.28 (0.22–0.37) and 2.33 (1.30–2.74), respectively. Elongated esophagus measuring 2.18 (1.56–2.77) length, divided into two portions where the first portion measures 0.98 (0.74–1.20) length and 0.17 (0.07–0.24) wide; the second portion measures 0.99 (0.42–1.37) length and 0.09 (0.05–0.19) wide. Esophageal bulb measuring 0.24 (0.17–0.36) in length and 0.21 (0.13–0.29) in width. Distance from vulva to anus and end of tail: 1.35 (1.00–1.94) and 1.69 (1.03–2.50), respectively. Tail measuring 300 (200–510) µm in length. Phasmids present. Thin-shelled eggs measuring 170 µm (100–980) length by 60 µm (50–80) wide.

Ozolaimus cirratus (based on light microscopy and scanning electron microscopy. Figures 5 to 8; Table 2)

Medium-sized parasite, rounded body with cuticle totally striated transversely. Dorsoventrally elongated buccal capsule with two lateral lips, and a triangular-shaped oral opening, and with serrated projections below the esophageal leaves. Esophagus long and divided into two portions: the first portion comprises the dilatation region, and the second portion is the one that goes to the bulb, the first portion being longer than the second. Bulb well developed; excretory pore immediately anterior or at the level of the bulb; nerve ring near the first portion of the esophagus. Males have a curved tail. Spicule long, distal end of the spicule is curved, and it is pointed. Genital cone present. Females with long uterus and vulva covered by a prominent vulvar lip. Gravid females thin-shelled eggs and no embryonated.

Based on 25 male specimens: body 5.95 mm (5.00–7.28) length and 0.49 (0.40–0.63) wide at the bulb region. Distances from anterior end to nerve ring and excretory pore 0.23 (0.17–0.29) and 1.77 (1.19–2.01), respectively. Elongated esophagus measuring 1.50 (1.12–2.15) in length, divided into two portions where the first measures 0.64 (0.52–0.74) in length and 0.17 (0.14–0.20) wide; the second measures 0.57 (0.36–0.72) in length and 0.09 (0.06–0.13) wide. Esophageal bulb measuring 0.20 (0.14–0.27) in length and 0.18 (0.11–0.20) in width.

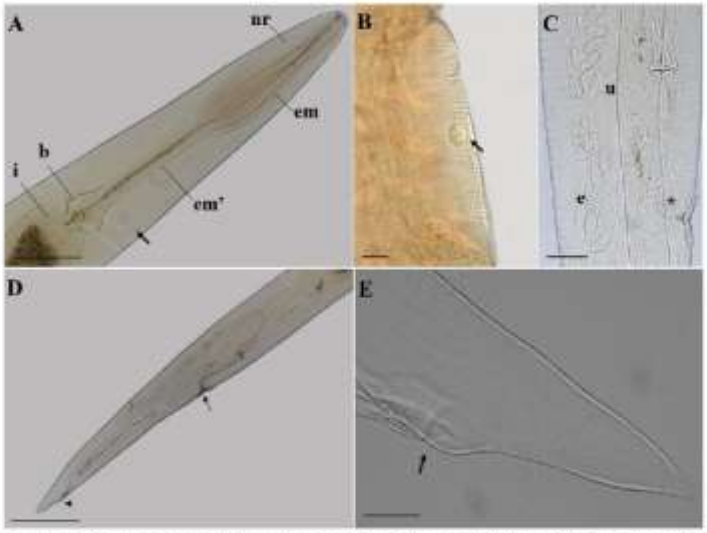

Figure 5. Light microscopy of female *Ozolaimus cirratus*, a parasite of *Iguana iguana*. A. Anterior extremity of the female, lateral view, nervous ring (nr), esophagus first portion (me), and muscular esophagus second portion (me'), prebulbar excretory pore (arrow), bulb (b) and intestine (i). Bar=300 µm. B. Excretory pore, lateral view (arrow). Bar=30µm. C. Vulvar region, note the short, muscular vagina (*), uterus (u) with eggs (e). Bar=100µm. D. Posterior end, lateral view, vulva (arrow) and anal opening (arrowhead). Bar=200µm. E. Posterior end, lateral view, anal opening (arrow). Bar=100µm.

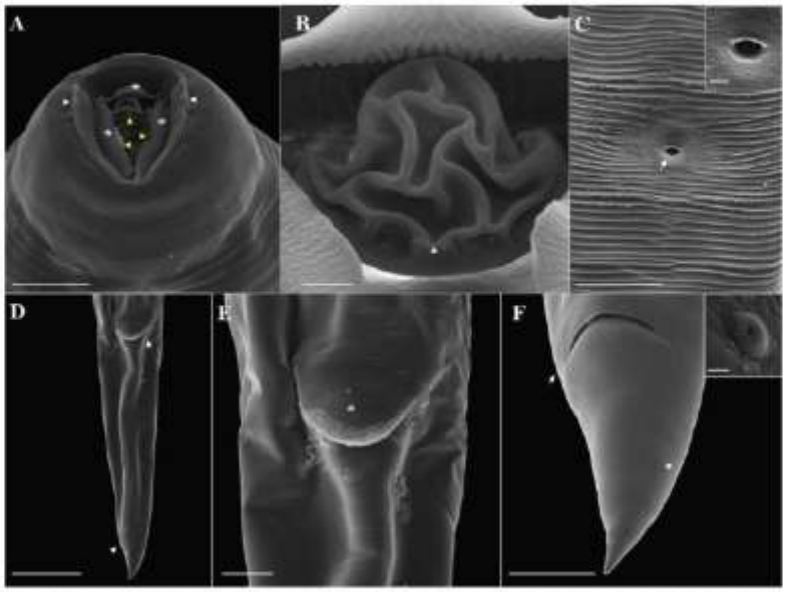

Figure 6. Scanning electron microscopy of female *Ozolaimus cirratus*, a parasite of *Iguana iguana*. A. Anterior end, projection of 3 tripartite cuticular membranes from the esophageal segment (*), amphids (arrowhead), opening of the esophagus into the oral cavity with esophageal projection (yellow arrowhead). Bar=50µm. B. Anterior end, esophageal leaves with serrated structures (arrowhead). Bar=5µm. C. Excretory pore (arrow). Bar= 50µm. Insert excretory pore opening. Bar=5µm. D. Posterior end, ventral view, vulva whose anterior lip projects slightly and protrudes the vulvar opening (arrow), and anal opening (arrowhead). Bar=500µm. E. Posterior end, ventral view, anterior lip of the vulva (*). Bar=100µm. F. Anal opening (arrow), and phasmid (arrowhead). Bar=100µm. Phasmid opening insert. Bar=2µm.

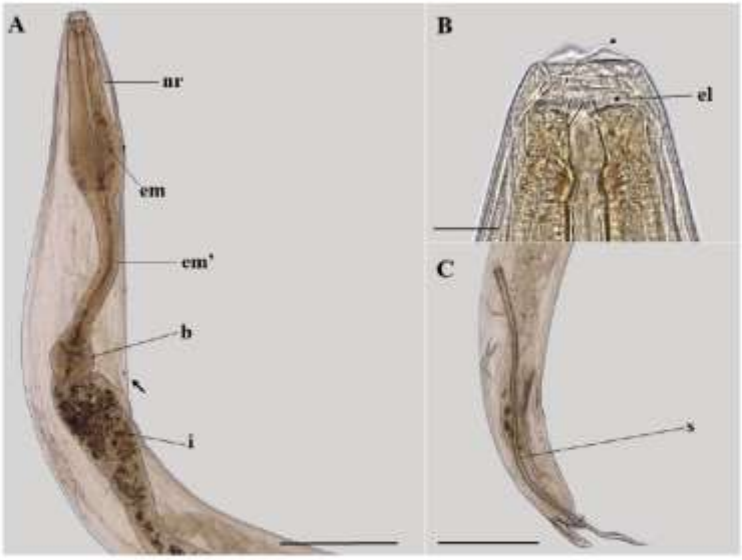

Figure 7. Light microscopy of male *Ozolaimus cirratus*, a parasite of *Iguana iguana*. A. Anterior extremity, nervous ring (nr), esophagus first portion (me), and muscular esophagus second portion (me'), prebulbar excretory pore (arrow), bulb (b) and intestine (i). Bar=500 µm. B. Lips (arrowhead), esophageal leaf (el). C. Posterior end, tail lateral view, spicule (s). Bar=500µm.

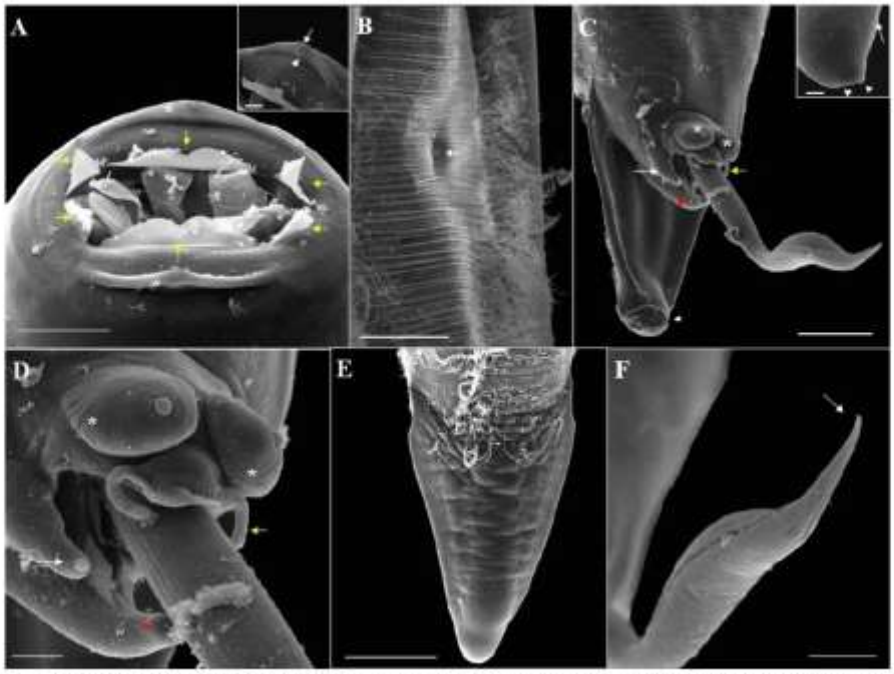

Figure 8. Electron microscopy of male *Ozolaimus cirratus*, a parasite of *Iguana iguana*. A. Anterior extremity in the cephalic region, a vertical slit mouth delimited by two conspicuous and hemispherical lateral lips (*), projection of six cuticular membranes (yellow arrow). Bar=20µm. Insert, lateral lip containing amphid (arrowhead), and orifice of the amphids canaliculus (arrow). Bar=5µm. B. Note the excretory pore (arrow). Bar=100µm. C-D. Posterior end, lateral and ventral view, two pairs of papillae can be observed, and a large pair (*), in a ventral and precloacal position and the other small pair located at the tip of the tail (arrowhead). Around the cloaca there are two pairs of appendages, one pair large and in the dorsolateral position (white arrow) containing a papilla at the end, genital cone containing two papillae (red arrowhead), and the other pair small membranous occupying a ventrolateral position (yellow arrow). Bar= 50µm. Insert caudal papillae (arrowhead) and phasmids (arrow). Bar= 5µm. E. Posterior end, tail dorsal view. Bar=50µm. F. Tip of the spicule. Bar=20µm.

A differentiated shape of the end of the spicule: spicule long, distal end of spicule curved, tapering to a point. Spicule 2.08 (1.50–2.37) in length. Tail measuring 170 µm (110–230) in length. Phasmids present.

Based on 20 female specimens: body 6.47 mm (4.46–7.77) length and 0.68 (0.53–0.86) wide at bulb region. Distances from anterior end to nerve ring and excretory pore 0.32 (0.29–0.37) and 2.19 (1.66–3.14), respectively. Elongated esophagus measuring 1.95 (1.55–2.80) in length, divided into two portions where the first portion measures 0.86 (0.71–1.22) in length and 0.22 (0.13–0.31) wide; the second portion measures 0.67 (0.50–1.00) in length and 0.10 (0.04–0.17) wide. Esophageal bulb measuring 0.26 (0.17–0.38) in length and 0.21 (0.17–0.38) in width. Distance from vulva to anus and end of tail: 1.39 (1.05–1.80) and 1.79 (1.42–2.22), respectively. Tail measuring 320 µm (170–510) in length. Phasmids present. Thin-shelled eggs measuring 110 µm (80–120) in length by 60 µm (50–70) in width.

Discussion

The genus *Ozolaimus* Dujardin, 1845, comprises medium-sized nematodes with a dorsoventrally elongated mouth with two lateral lips and a long esophagus divided into a short anterior portion and a thinner posterior portion ending in a distinct bulb. Side wings absent. Excretory pore at the anterior end; long spicule; short, truncated tail; well developed, curved distally. Females have a vulva anterior to the anus and covered by the vulvar lip, with a long and sinuous uterus (Rudolphi, 1819; Dujardin, 1845; Vicente et al., 1993). In our study, the morphological characters were compatible with this genus.

Five species of *Ozolaimus* parasites on lizards are currently described: *Ozolaimus megatyphlon* was initially described by Rudolphi (1819) found in the caeca of *Iguana iguana* in Berlin; *Ozolaimus cirratus* Linstow, 1906, in the large intestine of *Iguana tuberculata* Laurenti, 1768, in Germany; *Ozolaimus monhystera* (Linstow, 1902) in *Cyclura cornuta* (Bonnaterre, 1789) in Haiti; *Ozolaimus ctenosauri* Caballero, 1938, in the small intestine of *Ctenosaura pectinata* (Wiegmann, 1834) in Mexico; and *Ozolaimus linstowi* Malysheva, 2016 parasitizing the large intestine of *I. iguana* in Mexico. Of these authors, Linstow (1902, 1906) described the drawings referring to the two species *O. cirratus* and *O. megatyphlon* in more morphological detail. This was also the case with Ortlepp (1933), in his drawing of the esophagus and tail of these species. The images and descriptions contributed significantly to differentiating the species in our study. As observed in Table 1 of *O. megatyphlon* and Table 2 of *O. cirratus*, the morphometry of these species shows little difference between them, and in relation to other works, no significant differences were observed either.

In our research into the morphological analysis by LM, the species *O. cirratus* and *O. megatyphlon* differ from each other by the shape of the esophagus, position of the excretory pore, and shape of the spicule, as observed by Ortlepp (1933). And, for the first time through SEM, the presence of a small, serrated structure in the esophageal leaves of *O. cirratus* was described, while in *O. megatyphlon*, it presented small, spaced, and rounded structures in the esophageal leaves. In addition, phasmids were described for the first time in both species.

Parasites of this genus have been recorded in free-living iguanas in Midwest and Northeast Brazil (Breves et al., 2011; Teles et al., 2017; Otávio et al., 2018). The parasitological indices found in this work differ from those obtained by Teles et al. (2017), who obtained an average of approximately 1.600 *Ozolaimus* sp. per host. These parasites have also been recorded in iguanas in Peru by Arrojo (2002), in Panama by Bursey et al. (2007), and in Colombia and Suriname by Ávila & Silva (2010), demonstrating that these infections are very frequent. In the North region of Brazil, this was the first occurrence in the State of Pará, where 12.028 adult *O. megathyphlon* and *O. cirratus* were registered with a prevalence of 100% (n = 4), differing from the research by Otávio et al. (2018), which recorded 388 adult pinworms of *O. megatyphlon* and *O. cirratus* with a prevalence of 60% (n = 5), and Teles et al. (2017), which obtained a prevalence of 66.6% (n = 18), both in Brazil. There are still few records of *Ozolaimus* spp. in iguanids in Brazil.

The nematodes studied here, *O. megathyphlon* and *O. cirratus*, have also been recorded in *Iguana rhinolopha, Iguana tuberculate*, and *I. iguana* (Linstow, 1906; Dosse, 1942; Caballero, 1938; Loukopoulos et al., 2007; Breves et al., 2011; Otávio et al., 2018). Both Jacobson (2007) and Teles et al. (2017) report that the most common genera of *Iguana* parasites are *Alaeuris*, *Ozolaimus*, and *Tachygonetria*, and these pinworms have high host specificity, which is common in lizards, chelonians, and some snakes. The eggs, when ingested by the reptile, hatch in the upper digestive tract, which releases the larvae; when mature, adults migrate to the rectum (Greiner & Mader, 2006). They are usually elongated eggs with a flattened side, and most have a subpolar operculum (Anderson, 2000). The form of infection is mainly fecal-oral (Klingenberg, 2007). They are considered commensal organisms and can help in the digestion of foods of plant origin; however, in cases of massive infections, they can cause obstructions of the gastrointestinal tract, cloacal prolapse, and sometimes a slight local inflammatory reaction (Greiner & Mader, 2006; Klingenberg, 2007; Jacobson, 2007).

The iguanas in this research were free-living and had a high infection rate. According to van Marken Lichtenbelt (1993), free-living herbivorous lizards commonly present high loads of oxyurids, reaching up to 5.000 parasites per lizard. Kehoe et al. (2020) recorded an effective measure for controlling these helminths using oxfendazole to treat oxyurid nematodiasis.

Lent & Freitas (1948) identified a single site of infection in the genus *Ozolaimus* sp., the large intestine. Breves et al. (2011) also recorded infection by this nematode in the large intestine, and in coproparasitological and morphological analysis of adult helminths, Carvalho (2018) recorded *Ozolaimus* in the colon of a green iguana. In the present study, these nematodes were present throughout the large intestine, which corroborates the findings of the researchers mentioned above.

Conclusion

In this work, we report for the first time the infection by *O. megatyphlon* and *O. cirratus* in green iguanas in the state of Pará, thus expanding the geographical occurrence of the genus. Under scanning electron microscopy, we added distinguishing morphological characters between the two species, such as the presence of a small, serrated structure below the esophageal leaves in *O. cirratus* and small, spaced, and rounded structures below the esophageal leaves in *O. megatyphlon*. In addition to showing for the first time the phasmids in both species.

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Ethics declaration

Approval from research ethics committees was obtained to achieve the objectives of this study. No live animals were used in the study. Sisbio Protocol: nº 68028.

Conflict of interest

The authors declare that they have no conflict of interest.

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ARTIGO 5

Título: CARACTERIZAÇÃO MICROSCÓPICA DA RELAÇÃO PARASITO-HOSPEDEIRO ENTRE *Polymorphidae (Acanthocephala)* E *Phalacrocorax brasilianus* (Aves, Phalacrocoracidae) NA RESERVA EXTRATIVISTA MARINHA DE SOURE, PARÁ

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DOI:-

CARACTERIZAÇÃO MICROSCÓPICA DA RELAÇÃO PARASITO-HOSPEDEIRO ENTRE POLYMORPHIDAE (ACANTHOCEPHALA) DE *Phalacrocorax brasilianus* (AVES, PHALACROCORACIDAE) NA RESERVA EXTRATIVISTA MARINHA DE SOURE, PARÁ

RESUMO - Dentre os membros da família Phalacrocoracidae encontra-se *Phalacrocorax brasilianus*, ave aquática que possui hábitos alimentares piscívoros, podendo com isto, ingerir diversas fases infectantes de helmintos pertencentes a vários filos, como Acanthocephala, Platyhelminthes e Nematoda. Os indivíduos do filo Acanthocephala, quando adultos, realizam sua fixação ou ancoragem na mucosa intestinal de seus hospedeiros definitivos, podendo ou não produzir injúrias teciduais graves. A caracterização microscópica destas injúrias tem sido, ao longo dos tempos, demonstrada por meio de exames histopatológicos. Assim, após estudos em microscopia de luz obteve-se a descrição detalhada da interação tecidual parasito-hospedeiro entre acantocéfalos e *Phalacrocorax brasilianus*, fornecendo dados valiosos para aplicação no diagnóstico e monitoramento destas aves quando acolhidas em Centros de Triagem e Reabilitação de Animais Selvagens (CETRAS).

Palavras-chave: Amazônia; biguá; Histopatologia; Parasitologia.

INTRODUÇÃO

Phalacrocorax brasilianus (biguá) possui distribuição geográfica desde o sul dos EUA, passando pela América Central e toda América do Sul, apresentando hábitos migratórios (AVIBASE, 2023), sendo sua helmintofauna ainda não totalmente elucidada, bem como os processos imunopatologicos envolvendo os seus helmintos (MONTEIRO et al., 2011). As aves silvestres são importantes, pois mantêm o ciclo de vida dos helmintos, contribuindo com a manutenção e dispersão desses parasitos (BAHRAM et al., 2021).

Até o momento, os acantocéfalos que ocorrem parasitando o intestino de aves da família Phalacrocoracidae, são representados pelas espécies *Andracantha tandemtesticulata* (Monteiro, Amato & Amato, 2006) em Guaíba, Rio Grande do Sul – Brasil (MONTEIRO et al., 2006; MONTEIRO et al., 2011); *Andracantha phalacrocoracis* (Yamaguti, 1939) na Boêmia do Sul e Morávia do Sul - República Tcheca (MORAVEC; SCHOLZ, 2016) e Baía de São Vicente - Chile (GONZÁLEZ-ACUÑA et al. 2020); *Southwellina hispida* (Van Cleave, 1925) Witenberg, 1932, em Guanajuato - México (VIOLANTE-GONZÁLEZ et al., 2011) e Morávia do Sul - República Tcheca (MORAVEC; SCHOLZ, 2016); *Corynosoma arctocephali*

(Zdzitowiecki, 1984) em Baia de São Vicente - Chile (GONZÁLEZ-ACUÑA D et al., 2020); *Profilicollis altmani* (Perry, 1942) na Baia de São Vicente - Chile (GONZÁLEZ-ACUÑA D et al., 2020) em outras aves.

Membros do filo Acanthocephala estão associados a diversos níveis de mortalidade, principalmente em aves costeiras (SALA et al., 2013). Quando bem-sucedido, a transmissão ocorre quando o hospedeiro definitivo (Ex. Ave Phalacrocoracidae), ingere um cistacanto infectante encistado em artrópodes (moluscos, peixes etc.), que participam como hospedeiro intermediário e/ou paratênico, a depender da espécie envolvida, já no trato digestório das aves, esse grupo de helmintos, ainda imaturo, se instala no jejuno, e logo após a sua completa maturação sexual migra para o íleo (AZNARE et al., 2004; CABALLERO; SÁNCHEZ, 2022).

De acordo com Castro (1989) e Fairweather (1997) os acantocéfalos induzem alterações na estrutura do tecido intestinal (a nível de mucosa, muscular e serosa) e estas alterações afetam seu funcionamento normal. Baseando-se nessas informações, o objetivo desse estudo foi descrever a relação parasito-hospedeiro entre membros da família Polymorphidae (Acanthocephala) e a ave *Phalacocrorax brasilianus* (biguá) oriundos da reserva extrativista marinha de Soure-PA.

MATERIAL E MÉTODOS

Este trabalho faz parte integrante de tese de doutoramento na Pós-Graduação em Saúde e Produção Animal na Amazônia, onde o autor contribui como colaborador. A pesquisa possui licença do ICMBio/SISBIO nº 74195 e autorização do Comitê de Ética no Uso de Animais (CEUA-UFRA) nº 6309230520.

No período de 2020 a 2022, 20 espécimes de *Phalacrocorax brasilianus* foram obtidos da zona costeira do município de Soure (Reserva Extrativista Marinha de Soure) na Ilha de Marajó-Pará-Brasil (Latitude: -0,742862°, Longitude: -48,507732°). As aves são utilizadas como fonte alternativa de alimentação pelos pescadores da região, que gentilmente cederam as aves mortas que foram utilizadas neste estudo. Os animais foram individualizados em sacos plásticos e mantidos refrigerados em caixas isotérmicas com gelo para transporte até o Laboratório.

No laboratório, cada órgão foi individualizado em placa de Petri contendo solução salina de NaCl 0,9% e analisado em estereomicroscópio (Leica ES2), limpos, quantificados e armazenados em solução de AFA (93 partes de álcool etílico 70%, 5 partes de formaldeído,

2 partes de ácido acético glacial). Fragmentos de intestino contendo acantocéfalos aderidos foram coletados e fixados em formaldeído a 10% para análises histológicas. Os índices ecológicos de parasitismo foram analisados segundo Bush *et al.* (1997) e Bautista-Hernández *et al.* (2015).

Para MEV, os acantocéfalos fixados em A.F.A foram lavados em água destilada por 1 horas, pós-fixados em tetróxido de ósmio (O_3O_4) a 1% por 2 horas, sendo submetidos à desidratação em série crescente de etanol a partir do etanol 70% à 100% por 1 hora em cada bateria de álcool, posteriormente foram secos até ponto crítico de CO_2, montados em suporte metálicos de alumínio (*stubs*), metalizados com ouro+paládium e observados em microscópio eletrônico de varredura (VEGA 3, TESCAN) no Laboratório de Microscópio Eletrônica de Varredura, anexo ao LHEA, conforme Carvalho *et al.* (2022).

Fragmentos de intestino delgado pré-fixados, contendo parasitos aderidos a mucosa foram desidratados em concentrações crescentes de etanol 70%-100%, por 1 hora em cada bateria, clarificados em xilol em dois banhos de 30 minutos cada. Após isto, foi realizada a infiltração em parafina com três banhos sucessivos em parafina líquida por 20 minutos cada, em estufa a 60 °C, seguido de inclusão em histomoldes (TOLOSA *et al.*, 2003). Após a inclusão, os blocos foram seccionados em cortes de 4-5 µm de espessura, usando um micrótomo (ZEISS HYRAX M25). As lâminas obtidas foram coradas com Hematoxilina e Eosina, e Tricrômico de Gomory. As imagens foram obtidas em microscópio LEICA DM 2500], com sistema de captura digital acoplada (LEICA ICC50 HD) e software Leica Application Suite V4.4., onde foram obtidas fotomicrografias.

RESULTADOS E DISCUSSÃO

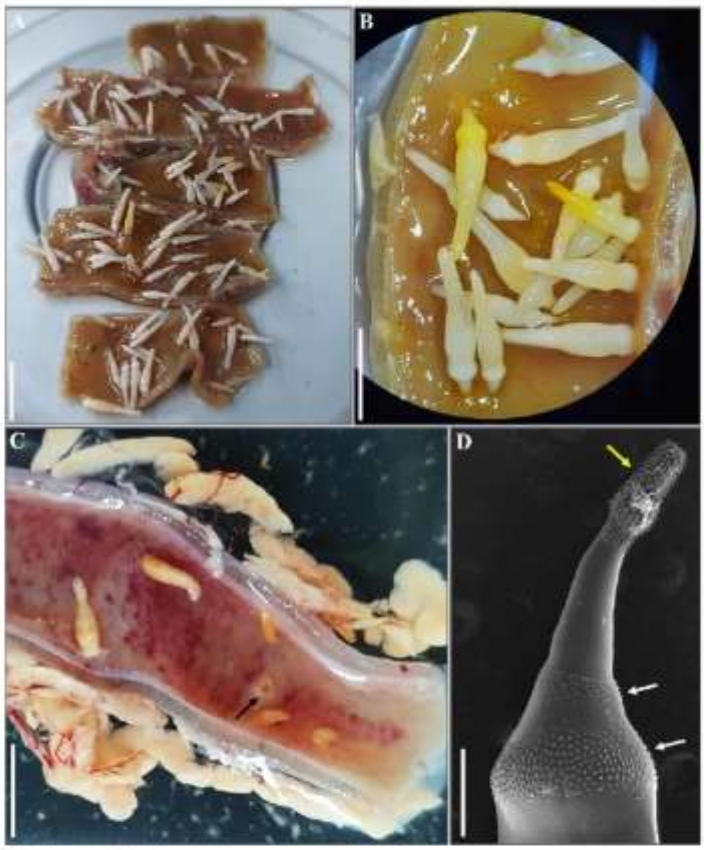
A resposta imune a agentes infecciosos é o principal fator que impede a ocorrência de infecções sistêmicas. Uma vez que as deficiências imunológicas na imunidade inata e adaptativa, são associadas a susceptibilidade a infecções virais, bacterianas, fúngicas e parasitárias. No que se refere aos helmintos, devido aos seus tamanhos variados e a diversidade metabólica, os mecanismos de respostas dos hospedeiros, são antigenicamente complexos (MACHADO *et al.*, 2004).

Forgiarini *et al.* (2016) em trabalho com galinhas poedeiras (*Gallus gallus*) afirmam que as infecções parasitárias em aves na maioria das vezes não causam grandes prejuízos ao desempenho produtivo. Já Dezfulie *et al.* (2002) afirmam que a extensão dos danos que os helmintos causam no intestino é proporcional a intensidade da infecção e da profundidade

de penetração do parasito nos tecidos do hospedeiro. Aznare *et al.* (2004) relata que os acantocéfalos jovens na maioria das vezes, são encontrados no jejuno e à medida que maturam sexualmente, os adultos migram para o íleo.

No presente estudo notou-se grandes áreas intestinais de *Phalacrocorax brasilianus* com intensa carga parasitária promovida por helmintos Polymorphidae (Figura 1), aderidos em diferentes níveis nas camadas mucosa, muscular e serosa intestinal, ocasionando uma enterite inflamatória e reações granulomatosas encapsulantes crônica de tecido fibroconjuntivo (enterite granulomatosa), semelhantes aos relatos de Sala *et al.* (2013) e Moore e Bell (1983) em estudos com corvo encapuzado (*Corvus corone cornix*), no entanto, mesmo nos casos mais severos, não foi observada peritonite difusa como nos relatos de Bahram *et al.* (2021) que trabalharam com corvo encapuzado e a pega-rabuda (*Pica pica*). As primeiras reações teciduais da relação parasito-hospedeiro evidenciadas no presente estudo, estão relacionadas a camada da mucosa (Figura 1), macroscopicamente foi observada extensa área hemorrágica na parede intestinal (Figuras 1C) e área com processo cicatricial evidente devido ao desprendimento do parasito (Figura 1C, seta preta).

Figura 1. (A) Fragmentos de intestino delgado de *Phalacrocorax brasilianus* com elevada carga parasitária promovida por acantocéfalos. **(B)** Em maior aumento, observa-se a fixação em diferentes níveis dos parasitos no intestino. **(C)** Evidencia-se extensa área hemorrágica na parede intestinal e área com processo cicatricial evidente devido ao desprendimento do parasito (seta preta). **(D)** Imagem de microscopia eletrônica de varredura de acantocéfalo, observa-se os ganchos da probóscide (seta amarela) e tronco com dois colares de espinhos (setas brancas).



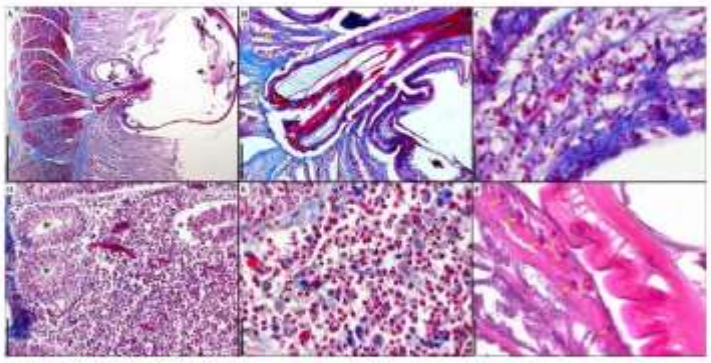
Fonte: acervo do autor

Microscopicamente, observou-se necrose das vilosidades intestinais (Figuras 2A, C), criptas (Figura 2D) e tecidos adjacentes, que segundo os trabalhos de Bahram *et al.* (2021) ocorrem devido a resposta do sistema imune do hospedeiro a injúrias causadas pelo metassoma e pelos ganchos da probóscide do parasito.

O parasitismo também ocasionou um processo inflamatório granulocítico (Figuras 2D, E, F), mediado por eosinófilos/ heterofilo (Figura 2C) e macrófagos (Figura 2E) que

conforme trabalho de Juul-Madsene *et al.* (2008) a formação do granuloma é uma importante resposta inata, que aumenta à medida que a ave amadurece. Além disso, um quadro hemorrágico na lâmina própria do tecido epitelial (Figura 2C), foi igualmente relatada em aves de rapina nas investigações de Santoro *et al.* (2010).

Figura 2. Cortes histológicos dos estágios iniciais da ancoragem de helminto Polymorphidae (Acanthocephala) na camada mucosa do intestino de biguá (*Phalacrocorax brasilianus*). **(A)** secção sagital do intestino, evidenciando a ancoragem na submucosa do acantocéfalo macho, identificado pela presença do testículo (seta vermelha); Observa-se a destruição das vilosidades do íleo próximas ao tegumento do parasito (seta preta). Corante Tricrômico de Gomori, barra de escala 500 µm. **(B)** Detalhe da ancoragem do parasito na muscular da mucosa, evidenciado os ganchos (seta verde) que auxiliam na fixação do helminto no tecido. Probóscide em eversão; chama a atenção a perda da arquitetura tecidual no local com discreta infiltração de tecido conjuntivo (*). Corante Tricrômico de Gomori, barra de escala 100 µm. **(C)** Vilosidade ileal com perda de sua arquitetura tecidual, observa-se a lise celular dos enterócitos e das células caliciformes, presença de processo hemorrágico e infiltrado eosinofílico (seta amarela). Corante Tricrômico de Gomori, barra de escala 20 µm. **(D)** Intenso processo inflamatório infiltrativo, chama a atenção os diferentes estágios infiltrativo e necrose das glândulas intestinais (*). Corante Tricrômico de Gomori, barra de escala 50 µm. **(E)** Micrografia da celularidade do processo inflamatório subjacente a ancoragem parasitária, evidenciando um quadro hemorrágico (setas pretas), com presença de infiltrado de granulócitos eosinófilicos (setas azuis) e presença de macrófagos polimorfos nucleados (setas verdes) e discretos neutrófilos (seta amarela). Corante Tricrômico de Gomori, barra de escala 20 µm. **(F)** Superfície de contato do metassoma do parasito com a mucosa intestinal, em destaque o intenso infiltrado eosinofílico (seta amarela). Corante hematoxilina e eosina, barra de escala 20 µm.

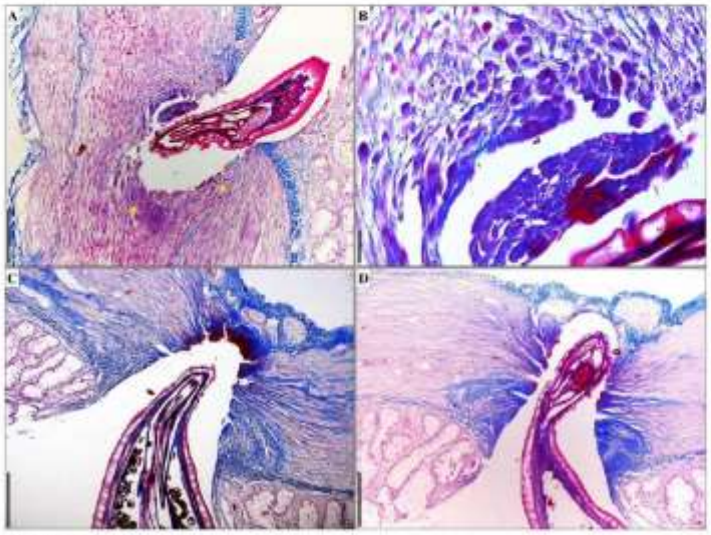


Fonte: acervo do autor.

Outro tipo de reação encontrada nesta pesquisa, foi a nível de camada muscular. O tecido conjuntivo cicatricial foi sendo depositado em torno dos helmintos à medida que estes ingressavam na camada muscular (Figuras 3A, C, D), além da presença de um agregado inflamatório, contendo hemácias e fibras conjuntivas em torno do gancho da probóscide, com infiltrado inflamatório no tecido adjacente (Figuras 3A, B) não sendo tão evidente o infiltrado de células granulocíticas (eosinófilos/ granulócitos), mas sim, áreas de necrose, hiperplasia e neoplasia tecidual em torno da probóscide do parasito, o quem em parte difere dos resultados encontrados por (SALA *et al.*, 2013) que não observaram abundantes células gigantes multinucleadas ao redor do parasito, estando estas restritas ao processo de cicatrização após a evasão ou a morte do parasito (Figuras 3D, E).

Figura 3. Cortes histológicos segmento intestinal (íleo) de biguá (*Phalacrocorax brasilianus*) parasitado por Polymorphidae (Acanthocephala) a nível de camada mucosa e muscular. **(A)** Secção sagital do intestino mostrando a ancoragem, na porção medial da camada muscular, é observada a presença de acúmulo de material amorfo azurofílico em contato com a probóscide do parasito, também é observada hiperplasia de células musculares (*), além de discreto infiltrado de tecido conjuntivo em azul. Corante Tricrômico de Gomori, barra de escala 200 µm. **(B)** Presença de um agregado inflamatório, contendo hemácias e fibras conjuntivas em torno do gancho da probóscide, também é observado infiltração inflamatório no tecido adjacente. Corante Tricrômico de Gomori, barra de escala 100 µm. **(C)** e **(D)** Fotomicrografias mostrando a sequência da perfuração

da probóscide até a camada serosa e aumento do tecido conjuntivo proporcional a transposição do parasito. Corante Tricrômico de Gomori, barra de escala 200 µm.

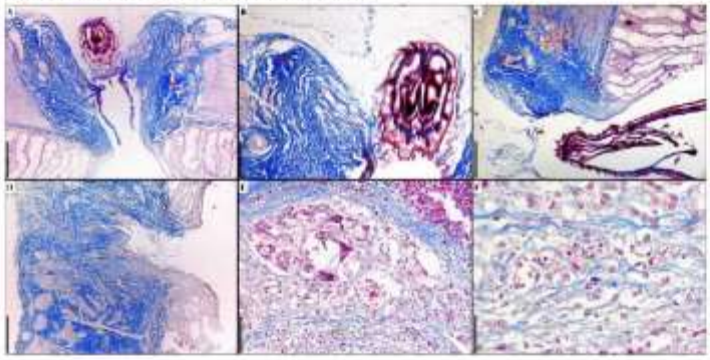


Fonte: acervo do autor.

Foi observado ainda, uma transfixação da parede intestinal pela probóscide do acantocéfala para a cavidade peritoneal da ave, sendo encapsulada por tecido conjuntivo fibroso (Figuras 4A, B, C). Isso permite que o parasito permaneça aderido ao intestino enquanto seu metassoma permaneça livre no lúmen para absorver os nutrientes (CABALLERO et al., 2020). Segundo (TARASCHEWSKI, 2000) áreas de necrose estão principalmente relaconadas ao tecido próximo ao corpo do parasito, juntamente com os processos neoplásicos, especialmente no peritônio.

Outro tipo de resposta observada, ocorre quando o parasito se destaca do local de fixação ou morre, havendo uma neoformação irregular do tecido muscular (Figura 4D), além de área circunscrita de reação granulomatosa crônica com grande quantidade de células polimorfo nucleadas, células gigantes, macrófagos e uma baixa quantidade de eosinófilos (Figura 4E, F).

Figura 4. Cortes histológicos sagitais de segmento intestinal (íleo) de biguá (*Phalacrocorax brasilianus*) parasitado por Polymorphidae (Acanthocephala) a nível de camada mucosa, muscular e serosa. **(A), (B) e (C)** Penetração profunda da probóscide na serosa parietal. É observado um pequeno feixe de fibra conjuntiva circundando a probóscide (seta preta), além de intensa deposição de tecido cicatricial (fibras conjuntivas) entorno do helminto com formação de áreas de necrose tecidual (*) não sendo possível descrever a celularidade. **(D), (E)** e **(F)** Fotomicrografias de áreas de reparação tecidual sem a presença do helminto, com intensa formação de tecido cicatricial em azul, com neoformação irregular do tecido muscular (seta amarela), além de áreas circunscrita de reação granulomatosa crônica com grande quantidade de células polimorfo nucleadas, células gigantes (setas pretas), macrófagos (setas vermelhas) e uma baixa quantidade de eosinófilos (setas verdes).



Fonte: acervo do autor.

CONCLUSÃO

Este é o primeiro registro histopatológico das alterações provocadas pelos helmintos do Filo Acanthocephala em *Phalacrocorax brasilianus* na Ilha de Marajó, no estado do Pará. A inserção da probóscide e ganchos de acantocéfalos no intestino delgado de biguá causou reações inflamatórias, hemorragia, necrose e destruição de vilosidades e criptas intestinais. Além disso, foi evidenciado que ocorre a perfuração total do intestino pela probóscide do acantocéfalo, sendo necessário mais estudos que investiguem as

consequências dessa injúria, uma vez que ainda são escassas as literaturas referentes as interações parasitos-hospedeiros em biguás.

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Orientação à Iniciação científica – Taxonomia de helmintos trematoda parasitos do sistema digestório de *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) na Ilha de Marajó, Pará

UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
PRÓ-REITORIA DE PESQUISA E DESENVOLVIMENTO TECNOLÓGICO
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PROIC CICLO 2021/2022

Elane Guerreiro Giese

William Wallacy Silva de Carvalho

TAXONOMIA DE HELMINTOS TREMATODA PARASITOS DO SISTEMA DIGESTÓRIO DE *Phalacrocorax brasilianus* (AVES: PHALACROCORACIDAE) NA ILHA DE MARAJÓ, PARÁ

Contribuição ao estudo morfofisiopatológico de primatas não humanos e demais animais silvestres da região Amazônica
(PIPA356-2009)

BELÉM-PARÁ

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Tese de Conclusão de Curso

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA - UFRA
INSTITUTO DE SAÚDE E PRODUÇÃO ANIMAL

GERÔNIMO DA SILVA CABRAL

CARACTERIZAÇÃO MICROSCÓPICA DA RELAÇÃO PARASITO-HOSPEDEIRO ENTRE Polymorphidae (Acanthocephala) E *Phalacrocorax brasilianus* (Aves, Phalacrocoracidae) NA RESERVA EXTRATIVISTA MARINHA DE SOURE, PARÁ

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2023

Resumos em eventos científicos: “IV Integra Ufra – Ciência, tecnologia e inovação na Amazônia Pós - pandemia”

CERTIFICADO

Certificamos que WILLIAM WALLACY SILVA DE CARVALHO submeteu o trabalho: OCORRÊNCIA DE NEMATODA ANISAKIDAE PARASITO DE Cairina moschata NA ILHA DE MARAJÓ, PARÁ, BRASIL na modalidade PARASITOLOGIA: WILLIAM WALLACY SILVA DE CARVALHO, ANDRESSA MALTA BRAULE PINTO, RICARDO LUIS SOUSA SANTANA3, ELAINE LOPES DE CARVALHO4, RAUL HENRIQUE DA SILVA PINHEIRO, ELANE GUERREIRO GIESE5 trabalho apresentado no evento "IV INTEGRA UFRA - “CIÊNCIA, TECNOLOGIA E INOVAÇÃO NA AMAZÔNIA PÓS- PANDEMIA” realizado entre os dias 26 de julho à 30 de julho de 2021 na Universidade Federal Rural da Amazônia (UFRA).

Coordenação do Programa de Iniciação Científica

Pró-reitoria de Pesquisa e Desenvolvimento Tecnológico

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