MORPHOLOGICAL, MOLECULAR AND ECOLOGICAL INTEGRATIVE TAXONOMY OF ACANTHOCEPHALA (ARCHIACANTHOCEPHALA) PARASITE OF BRAZILIAN WILDLIFE MAMMALS

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INSTITUTO OSWALDO CRUZ
Programa de Pós-Graduação em Biologia Parasitária

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Morphological, molecular and ecological integrative taxonomy of Acanthocephala (Archiacanthocephala) parasite of Brazilian wildlife mammals

Tese apresentada ao Instituto Oswaldo Cruz como parte dos requisitos para obtenção do título de Doutor em Ciências

Orientador (es): Prof. Dr. Arnaldo Maldonado Júnior
Prof. Dra. Natalie Olifiers

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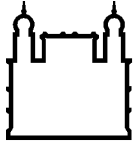
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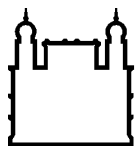
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Anexar a cópia da Ata que será entregue pela SEAC já assinada.

Dedico a minha família, aos meus pais Maria Natividade e Sérgio Otávio, a minha irmã Cecília e meu namorado Erick que sempre me apoiaram e acreditam em mim.

3

AGRADECIMENTOS

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"There is a driving force more powerful than steam, electricity and nuclear power: the will" Albert Einstein

"Há uma força motriz mais poderosa que o vapor, a eletricidade e a energia atômica: a vontade" Albert Einstein

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TAXONOMIA INTEGRATIVA, MORFOLÓGICA, MOLECULAR E ECOLÓGICA DE ACANTHOCEPHALA (ARCHIACANTHOCEPHALA) PARASITOS DE MAMÍFEROS SILVETRES BRASILEIROS

RESUMO

TESE DE DOUTORADO EM BIOLOGIA PARASITÁRIA

Ana Paula Nascimento Gomes

O filo Acanthocephala é caracterizado por não possuir trato digestório e por apresentar na região anterior uma probóscide munida de ganchos que retrai-se para dentro de um receptáculo. Este grupo é dividido em quatro classes Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala e Polyacanthocephala baseado em características morfológicas, biológicas e ecológicas. Dentre os filos dos helmintos estudados em mamíferos brasileiros, o filo Acanthocephala se destaca por apresentar lacunas no que se refere às informações taxonômicas, filogenéticas e ecológicas. O objetivo geral deste trabalho foi realizar a taxonomia integrativa dos acantocéfalos recuperados em mamíferos das famílias Procyonidae, Myrmecophagidae e Cricetidae de diferentes regiões geográficas do Brasil, armazenados e disponibilizados pela coleção do Laboratório de Biologia e Parasitologia de mamíferos Silvestres Reservatórios (LABPMR) utilizando características morfologicas, moleculares e ecológicas. Os acantocéfalos recuperados foram identificados através da microscopia de luz (ML) e por microscopia eletrônica de varredura (MEV). Foi também realizada a análise filogenética molecular dos acantocéfalos com os marcadores moleculares do gene ribossomal da subunidade maior (28S rRNA) e do gene mitocondrial citocromo oxidase da subunidade 1 (MT-CO1). Além disto, foi determinada a prevalência e abundância dos ovos de Acanthocephala através da análise coproparasitológica de fezes de quati *Nasua nasua* e de cachorro-do-mato *Cerdocyon thous*, avaliando a influencia dos fatores bióticos e abióticos na infecção. Os espécimes de acantocéfalos foram descritos e identificados em duas novas espécies *Pachysentis* n. sp. (Archiacanthocephala: Oligacanthorhynchidae) parasitando *Nasua nasua* (quati) proveniente do Mato Grosso do Sul do bioma Pantanal e *Moniliformis n. sp.* (Archiacanthocephala: Moniliformidae) em *Necromys lasiurus* (ratinho-do-cerrado) da região de Uberlândia, Minas Gerais do bioma Cerrado; e redescrita a espécie *Gigantorhynchus echinodiscus* (Archiacanthocephala: Gigantorhynchidae) em *Myrmecophaga tridactyla* (Tamanduá-bandeira) da Estação Ecológica Santa Bárbara, São Paulo, bioma cerrado. As análises filogenéticas moleculares sugeriram que a espécie *G. echinosdichus* está relacionada com *Mediorhynchus* sp. formando um grupo monofilético, assim como *Moniliformis* n. sp. está relacionado com as espécies do gênero *Moniliformis* também formando grupo monofilético. A análise ecológica foi realizada com 118 amostras fecais de 55 espécimes de cachorro-do-mato e 72 amostras fecais de 61 espécimes de quatis sugerindo a influência da sazonalidade na abundância dos acantocéfalos para ambos os hospedeiros e que os atributos relacionados ao hospedeiro como sexo e idade também constituíram fatores importantes associados à prevalência e às cargas parasitárias. O presente trabalho acrescentou informações morfológicas, moleculares e ecológicas, enfatizando a importância de adotar abordagem da taxonomia integrativa nos estudos com Acanthocephala.
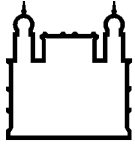
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ABSTRACT

PHD THESIS IN PARASITE BIOLOGY

Ana Paula Nascimento Gomes

The phylum acanthocephala is characterized by the presence of a proboscis armed with hooks, which retracts into receptacle, and lack of alimentary tract. This group is divided in four classes Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala based on morphological, biological and ecological characteristics. Among the helminths studied in Brazilian mammals, the phylum Acanthocephala have a lack of taxonomic, phylogenetic and ecological information. The aim of the present work was to perform the integrative taxonomy of acanthocephalans recovered in mammals of the family Procyonidae, Myrmecophagidae and Cricetidae from different geographic regions, store and made available by the Laboratory of Biology and Parasitology of Wild Reservoirs Mammal (LABPMR) using morphological, molecular and ecological characteristics. The recovered acanthocephalans were identified by light microscopy (ML) and by scanning electron microscopy (SEM). In addition, molecular phylogenetic analyses of the acanthocephalans was performed with the molecular markers of ribosomal large subunit (28s rRNA) gene and mitochondrial cytochrome c oxidase subunit 1 (MT-CO1). Furthermore, the prevalence and abundance of acanthocephala's eggs were determined by coproparasitological analyses of brown-nosed coatis *Nasua nasua* and crab-eating fox *Cerdocyon thous*, evaluating the influence of biotic and abiotic factors on infection. The acanthocephalan specimens from the LABPRM collection were analyzed, and two new species were described and identified: *Pachysentis* n. np. (Archiacanthocephala: Oligacanthorhynchidae) parasitizing *Nasua nasua* (brown-nosed coati) from Mato Grosso do Sul in the Pantanal wetland, and *Moniliformis n. sp.* (Archiacanthocephala: Moniliformidae) parasitizing *Necromys lasiurus* (hairy-tailed bolo mouse) from Uberlândia in the state of Minas Gerais in the cerrado biome; and one species were redescribed *Gigantorhynchus echinodiscus* (Archiacanthocephala: Gigantorhynchidae) in *Myrmecophaga tridactyla* (giant anteater) from Santa Bárbara Ecological Station, state of São Paulo in the cerrado biome. Molecular phylogenetic analyses suggested that *G. echinosdichus* is related to *Mediorhynchus* sp. forming a monophyletic group, as well as *Moniliformis* n. sp. is related to the species of the genus *Moniliformis* also forming a monophyletic group. The ecological analysis was performed with 118 fecal samples of 55 specimens of crab-eating fox, and 72 fecal samples of 61 specimens of coatis, and suggested the influence of seasonality on the abundance for both hosts; as well as the attributes related to the host as sex and age were important factors associated with prevalence and parasitic load. The present work added morphological, molecular and ecological informations, emphasizing the importance of adopting integrative taxonomic approaches in studies on acanthocephala.

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1   INTRODUCTION

1.1 Integrative Taxonomy

The central role of taxonomy is to generate biological information to characterize, classify and name taxa, aiming to explore and understand biodiversity (Sukumaran and Gopalakrishnan, 2015). It has helped the progress of species definition and characterization in the last decade (Wiens, 2007).

Currently, the taxonomy of recent groups integrates several disciplines for species determination and delimitation. The results come from information on population biology, mating behavior, morphology, genetics, molecular phylogeny, and phylogeography, all of which can contribute to species delimitation and consequently have been used in integrative taxonomy. Dayrat (2005) defined integrative taxonomy as a science in the early 2000s. He proposed this term to denote a comprehensive approach to delimit, name and, describe taxa by integrating information from different disciplines and using various methods. For example, some studies have connected morphological diversity and molecular phylogeny (e.g., Yeates et al., 2010) while others have combined morphological, molecular and chemical data to identify species (e.g., Heethoff et al., 2011).

In the scope of helminthology, the taxonomy used morphologic and morphometric data for species identification by microscopy tecnique. Currently, the taxonomy of recent groups integrate several disciplines for the construction of a complex of factors associated with the determination of a species. Modern taxonomic pratices in helminths parasites have been combined morphological and molecular data to description and characterisation species. Molecular tools offer an opportunity to include new components in discovery and description of parasite biodiversity (Nadler and Pérez-Ponce de León, 2011).

An integrative approach to taxonomy is necessary because the complexity of species biology requires a multiple and complementary approach. In addition, the level of confidence in identification of species supported by different kinds of data is much higher than for species supported by only one kind. Applying this integration can be a challenge to taxonomists and requires collaboration among multiple disciplines.

1.2 Phylum Acanthocephala

1.2.1 Morphology and Classification

Acanthocephala (Greek akantha = hook, kephale = head) are a small and monophyletic phylum which has around 1,300 obligatory endoparasite species. The name of the phylum refers to the helminth's organ for attachment to the host intestine, commonly known as a proboscis. These parasites are globally distributed and can be found in marine, freshwater or terrestrial hosts, in all biomes (Bush et al., 2001; Kennedy, 2006). The phylum is divided into four classes: Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala (Amin, 1987a, 2013), based on morphological, biological and ecological characteristics such as the number and shape of the cement glands; size and arrangement of proboscis hooks; intermediate and definitive host types; and host ecology (Bullock, 1969; Amin, 1985; Kennedy, 2006).

Archiacanthocephala are strictly terrestrial, using insects and myriapods as intermediate hosts and mammals and birds as definitive hosts. In some cases, however, they use reptiles and amphibians as paratenic hosts, remaining in the larval stage until reaching an appropriate definitive host (Schmidt, 1985; Kennedy, 2006). In contrast, the Palaeacanthocephala are mostly aquatic, having aquatic arthropods as intermediate hosts, and showing a high diversity of definitive hosts such as fish, birds or mammals that have a connection with aquatic habitats. Paratenic hosts are not very common, but this class still shows great diversity in terms of definitive hosts (Near et al., 1998; Kennedy, 2006). On the other hand, representative species of the Eoacanthocephala encompass aquatic species using crustaceans such as copepods and ostracodes as intermediate hosts and fish, amphibians and reptiles (especially turtles) as definitive hosts (Kennedy, 2006). Polyacanthocephala compose a small and isolated aquatic group, with one order, one family, one genus, and four species. Three species infect caimans (Alligatoridae) as definitive hosts in South America and one species is known to infect freshwater fish in Kenya and South Africa (Amin, 1985, 1987b; Amin and Dezfuli, 1995; Kennedy, 2006). These helminths are characterized by the presence of a proboscis armed with hooks; a lacunar system, a directional-flow circulatory system, with channels to promote direct absorption of nutrients and act as the motive force for fluid flow through the body wall; and lack of alimentary tract (Smyth, 1994). Acanthocephalans have a proboscis with hooks, a

neck and a trunk. In general, in the anterior end in both sexes (*praesoma*) the proboscis is armed with hooks that are used for attachment to the intestinal wall of the definitive host, which can cause some damage such as chronic enteritis with ulcerative lesions (Dunn, 1963; Muller et al., 2010). The neck is an unspined and smooth area between the posterior and distal hooks of the proboscis, and is the first infolding of the body wall. The proboscis is variable in shape and is covered by a tegument within which are embedded the roots of the sclerotized hooks, being able to retract into a structure called the receptacle (Travassos, 1917; Crompton and Nickol, 1985) (Figure I). At the base of the receptacle there is the cerebral ganglion, which associates with the peripheral nervous system. At the base of the neck, at the end of the proboscis, are the lemnisci (Figure I), which are involved in the fluid flow in relation to the proboscis movement. In the posterior region (*metasoma*) or trunk are the reproductive organs (Smyth, 1994; Bush et al., 2001).

Acanthocephalans are dioicous and exhibit marked sexual dimorphism, with the females usually being larger than the males. Reproduction is exclusively sexual and polygamy is frequent, with one male being able to fertilize several females (Smyth, 1994). Reproductive organs of males are formed by two testicles, and two other accessory organs: the cement glands and the copulatory bursa (Figure I). There can be one to eight cement glands, which secrete a substance called cement that is passed to the ejaculatory canal and can be stored in a reservoir. The secretions of cement glands when released are used for the formation of copulatory caps, to close the female's gonopore and sometimes that of males (Amin, 1985; Smyth, 1994; Núñez and Drago, 2017). The copulatory structures consist of the muscular Saefftigen's pouch, the eversible campanulate bursa, and the penis. The bursa everts during copulation and spreads over the posterior extremity of the female, followed by attachment (Figure I) (Amin, 1985; Bush et al., 2001; Núñez and Drago, 2017). In females, there is a complex apparatus composed of gonads from which ovarian balls develop to produce oocysts; the ligament sac, which contains the developing eggs; and an efferent duct, comprising a uterine bell, uterus and vagina (Figure I) (Amin, 1985; Bush et al., 2001). Fertilized females have eggs in the body cavity, and mature (embryonated) eggs are composed of four membranes, and are selected by the bell, which allows them to pass through the uterus and vagina and be released only when they are fully mature, with the fully-formed acanthor larva (Amin, 1985; Bush et al., 2001; Núñez and Drago, 2017).

The structures and organs in acanthocephalan specimens are also used in the taxonomy and diagnosis of the species, such as size and shape of the body; proboscis shape; size, shape and number of proboscis hooks; length of lemnisci; size, shape and position of the testicles; size and number of cement glands; and shape and size of the eggs (Amin, 1985).
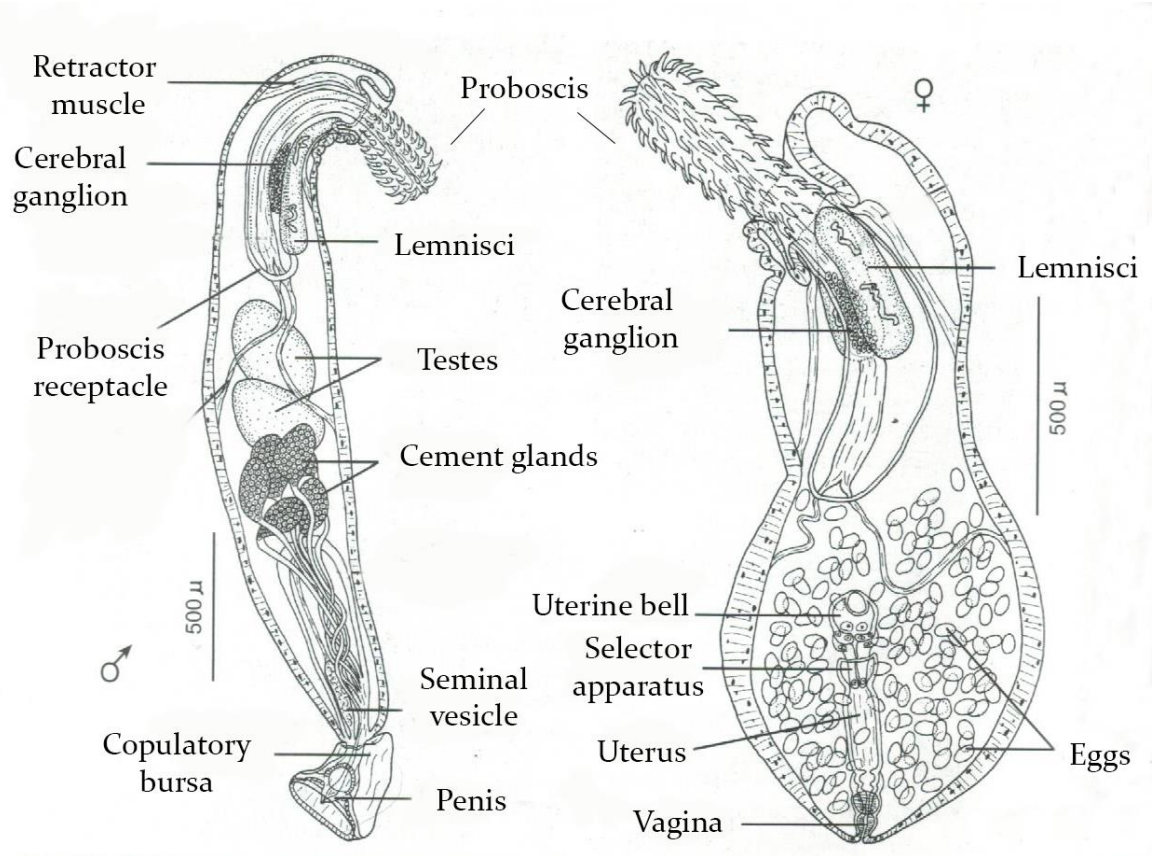
Figure I. Morphology of adult acanthocephalans: male and female (Adapted from "Parasitism: the diversity and ecology of animal's parasites" by Bush et al., 2001)

1.2.2 Life Cycle

Acanthocephalans have a complex and indirect life-cycle, by exploiting trophic interactions between arthropods and vertebrates (Read, 1974; Crompton and Nickol, 1985). Mature eggs are released by the female acanthocephalan into the vertebrate definitive host's gut and exit the host in feces (Kennedy, 2006; Santos et al., 2013) (Figure II). Rarely, an entire gravid female may be released with the feces, and the eggs then released during decay of the adult body (Kennedy, 2006). The shelled acanthor emerges from the egg after being ingested by a suitable intermediate host, penetrates the intestinal wall, and attaches to the hemocele, where it develops into an acanthella and then into a cystacanth, the infective stage to the vertebrate definitive host. Completion of the life cycle, including reproduction, occurs when an appropriate vertebrate definitive host ingests an infected arthropod intermediate host with the cystacanth (Figure II) (Conway Morris and Crompton, 1982; Amin, 1985; Schmidt, 1985; Santos et al., 2013). In addition, in unsuitable hosts, the eggs may be unable to hatch, so they pass out in the host's feces, or the acanthella may be unable to penetrate the intestinal wall or develop.

Occasionally, vertebrates may also serve as paratenic hosts, in which the acanthocephalan larvae (cystacanths) move to the body cavity of the vertebrate and attach to the mesenteric organs, where they encyst until ingested in the body cavity by a definitive host (Nickol, 1985). Paratenic hosts bridge the trophic level between intermediate and definitive hosts (Bush et al., 2001).

All species of acanthocephalans have the same larval stages and require only a single intermediate host, according to the species involved in the life cycle. For example, a terrestrial intermediate host can be a beetle or cockroach if the definitive host is terrestrial animal such as a bird or a mammal; or it may be a crustacean if the definitive host is a freshwater or marine species (Kennedy, 2006). However, cases of human infection are rare and accidental, being recorded by only seven species (Nicholas, 1967; Haustein et al., 2010) for example, *Moniliformis moniliformis*, *Macracanthorhynchus hirudinaceus*, *Macracanthorhynchus ingens*, *Acanthocephalus rauschi*, *Pseudoacanthocephalus bufonis*, *Corynosoma strumosum*, *Bolbosoma* sp. (Dingley and Beaver, 1985; Muller, 2002; Sahar et al., 2006; Berenji et al., 2007; Arizono et al., 2012).
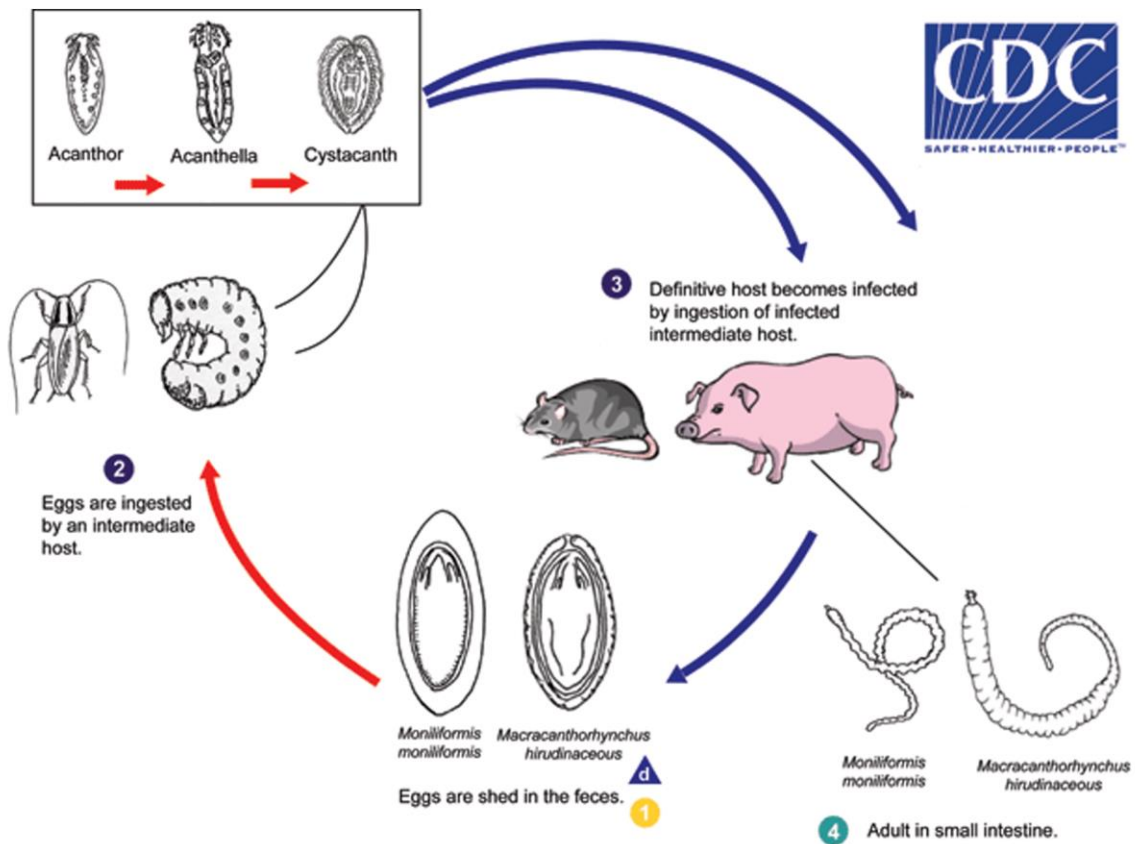
Figure II. Life cycle of acanthocephalans infecting terrestrial hosts (Center for Disease Control and Prevention, CDC). 1- Eggs are shed in the feces of the definitive hosts; 2- Eggs ingested by intermediate hosts (insect) develop into three larval stages; 3- Intermediate host infected by a cystacanth and ingested by definitive host; 4- Male and female adult acanthocephalans in the intestine of definitive hosts. (http://www.cdc.gov/dpdx/acanthocephalisis/index.html).

1.2.3 Ecological traits

The helminth parasites have a variety of transmission patterns and ecological requirements, and several factors can influence host-parasite relationship and host-environmental interaction (Mas-Coma et al., 2008). Parasitic infections are influenced by biotic factors such as host age, species, food habits, habitat, gender and physiological condition, as well as abiotic factors, such as seasonality, temperature and humidity. Therefore, biotic and abiotic factors can influence prevalence, intensity and abundance of helminths (Poulin, 1999; Arneberg, 2001; Poulin, 2006). Recent studies have reaffirmed an evidence of the relationship between ecological factors and the number of endoparasites, richness and structure of the helminth community in several hosts (Lindenfors et al., 2007; Simões et al., 2011; Cardoso et al., 2016; Castro et al., 2017; Spickett et al., 2017).

According to Kennedy (2006), seasonal variation such as rainfall and temperature, and factors related to the host diet in different geographic regions in the world, have a strong correlation with prevalence and abundance of infection in different species of the classes Eoacanthocephala and Palaeacanthocephala. Environmental features such as water temperature, and infection patterns of acanthocephalans in intermediate hosts (crustaceans and isopods) and definitive hosts (fish, birds and aquatic mammals) have been associated with maturation of acanthocephalan larvae, as well as to the prevalence, abundance and intensity of the infection.

In addition, Amin (1987b) and Amin et al (2008) and Rauque et al (2006) showed that the infection patterns are influenced by the feeding habit of the definitive hosts. They verified that the prevalence and intensity of acanthocephalans in the definitive hosts were affected by seasonal changes, were peaked in summer and autumn due to the recruitment of acanthocephalans and low in the winter due to the lower temperature. Thus, these authors attributed the infection rates to the feeding habits of vertebrate definitive hosts. Liat and Pike (1980) reported the occurrence of *Profilicollis botulus* (Van Cleave, 1916) Witenberg, 1932 in the duck *Somateria mollissima* (Linnaeus, 1758), and attributed the higher levels of infection in young ducks to the consumption of the crab *Carcinus maenas*. However, the intensity declined with the age due to diet change. Recently, Lisitsyna et al. (2018) showed that the prevalence and intensity of *Corynosoma strumosum* (Rudolphi, 1802) Lühe,

1904 and *C. obtuscens* Lincicome, 1943 were related with the age class of sea lions in California due to change in feeding habits.

Ecological studies of acanthocephalans regarding the influence of biotic factors such as host age, sex or size on patters of infection have also been performed. Amin (1987b) studied fish species in Wisconsin lakes as definitive hosts of *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 and did not find a correlation between the acanthocephalans and the age and size of the definitive hosts. However, he found a difference between host genders.

Although many studies have been performed with aquatic arthropods and aquatic vertebrates (Liat and Pike, 1980; Amin, 1984; Sinisalo et al., 2004; Kennedy, 2006; Steinauer et al., 2006; Franceschi et al., 2008; Amin et al., 2008; Caddigan et al., 2014; Amin, 2016), there is a lack of research on the ecology of acanthocephalans of terrestrial mammals. Thus, ecological studies are important to understand the dynamic of infection of acanthocephalans and the relationship with their hosts, especially for terrestrial vertebrates such as mammals.

1.2.4 Molecular phylogeny

The history of the Acanthocephala classification consists of taxonomic studies based mainly on morphological methods. Recently, molecular approaches with DNA sequencing using different molecular markers have complemented the conventional taxonomic work. Molecular biology studies have separated sibling species, revealing cryptic diversity, and have unambiguously identified eggs, larvae, females and fragments of parasites to the species level, as well as investigating inter and intraspecific genetic variation within acanthocephalan species (Near et al., 1998; Near, 2002; García -Varela and Nadler, 2005; García-Varela and Pérez-Ponce de León, 2015; Pinacho-Pinacho et al., 2015; Wayland et al., 2015).

Molecular biology has also been used to make phylogenetic inferences between taxa. The most frequent molecular markers used in phylogenetic studies of Acanthocephala are the small subunit (SSU) or 18s rRNA gene and the large subunit (LSU) or 28S rRNA gene, both of which are ribosomal RNA genes (rRNA) (García-Varela and Pérez-Ponce de León, 2015). These markers began being used in the 1990s to elucidate the relationships among the four classes within the phylum Acanthocephala, showing that the phylum is a monophyletic group. The Archiacanthocephala class is a sister taxon of the Palaeacanthocephala and

Eoacanthocephala classes, whereas the Polyacanthocephala class forms a sister group with Eoacanthocephala (Near et al., 1998; Near, 2002; García-Varela and Nadler, 2006). In addition, those studies inferred the phylogenetic relationship between Rotifera (free-living aquatic organisms belonging to the zooplankton in the limnetic community) and Acanthocephala and other pseudocelomates (Near et al., 1998; García- Varela et al., 2000, 2002; Herlyn et al., 2003). These findings provide strong support for the existence of a clade including Rotifera plus Acanthocephala (so-called Syndermata), and support the hypothesis that the acanthocephalans share a more recent common ancestor with Rotifera (Garey et al., 1996; Melone et al., 1998; Giribet et al., 2000; Near, 2002).

Recently, molecular phylogenetic studies of acanthocephalans have incorporated other markers such as the two internal transcribed spacer regions (ITS1 and ITS2) separated by the 5.8S rRNA gene, forming the complex ITS1-5.8S rRNA-ITS2 - (Complex-ITS) and mitochondrial cytochrome c oxidase subunit I (MT-CO1). According to García-Varela and Pérez-Ponce de León (2015), phylogenetic studies carried out with ITS-complexes have shown that these genes can be used to establish species boundaries within some genera, due to relatively variable regions within species. Studies have also shown inter and intraspecific genetic variation in some genera such as *Pomphorhynchus* Monticelli, 1905, *Profilicollis* Meyer, 1931, *Echinorhynchus* Zoega in Müller, 1776, *Leptorhynchoides* Kostylew, 1924, *Neoechinorhynchus* Stiles et Hassall, 1905, and *Corynosoma* Lühe, 1904, explaining that most of the variation results from the presence of cryptic species (Král'ová - Hromadová et al., 2003; García-Varela et al., 2005; Steinauer et al., 2006; Pinacho-Pinacho et al., 2015). Cryptic species are two or more species that have been classified as single nominal species because they are morphologically indistinguishable, not biologically similar but genetically distinguishable (Bickford et al., 2007). Molecular techniques (DNA sequencing) have transformed the ability of scientists to describe and define biological diversity (Bickford et al., 2007). The mitochondrial cytochrome c oxidase subunit I (MT-CO1) is one of the most frequently used molecular markers for population genetics and phylogeographic studies across multiple divergent taxa. In acanthocephalans, it has been used to reformulate hypotheses of phylogenetic relationships and to recognize and establish species limits (Guillén-Hernández et al., 2008; Alcántar-Escalera et al., 2013; García-Varela et al., 2013).

Molecular phylogenetic analysis and classical systematic phylogeny have contributed to understand the classification of acanthocephalans; to establish relationships between different hierarchical levels, such as classes, families and genera; to define biological diversity, establishing limits between species; and to understand life cycles, such as the roles of larvae and adults in their respective intermediate and definitive hosts. Furthermore, molecular and phylogenic studies help to resolve evolutionary and ecological questions, such as: a) the evolutionary relationship between the phylum Acanthocephala and rotifers, suggesting that they are sibling taxa; b) the evolution of parasitism within the group; and c) the life cycles and pattern of association of acanthocephalans with their arthropod intermediate hosts and vertebrate definitive hosts (Backeljau et al., 1993; Raff et al., 1994; Winnepenninckx et al., 1995; Near et al., 1998; Near, 2002).

1.2.5 *Acantocephala from Brazilian Wildlife Mammals*

Travassos (1917) reviewed Brazilian acanthocephalans and concluded that Brazilian Gigantorhynchida was a taxon with great diversity, including around 40% of the species, which now compose the orders Oligacanthorhynchida, Moniliformida and Gigantorhynchida. Later, Amin (2000) compiled and reviewed the acanthocephalans from the Neotropical region, correlating the distribution of species with the distribution of the scientists studying them. He observed a large number of endemic genera and species of acanthocephalans in South America, with most of them being well studied in Brazil (for instance, by Travassos, Machado Filho and Salgado-Maldonado). Furthermore, he emphasized that most genera described in South America have been reported in Brazil in different hosts.

The history of the investigation of Acanthocephala in Brazil started in the early twentieth century with Dr. Lauro Travassos, a parasitologist from *Fundação Oswaldo Cruz* (Oswaldo Cruz Foundation), who carried out taxonomic reviews of genera and families of Brazilian acanthocephalans, and Dr. Domingos Machado Filho, who was a pupil of Dr. Travassos and described numerous genera and species for the taxa. Since then, several manuscripts about Brazilian Acanthocephala from vertebrates in different geographic regions have been reported (Gomes et al., 2015; Macedo et al., 2016; Catenacci et al., 2016; Muniz-Pereira et al., 2016; Santos and Gibson, 2015; Santos et al., 2017; Souza et al., 2017). Currently, 46 species of acanthocephalans infecting different orders of mammals are known (Figure III). The Carnivora and

Primates are the orders most frequently found infected, respectively with 23 acanthocephalan species in 19 carnivore hosts and 10 acanthocephalan species in 11 primates. On the other hand, few species of acanthocephalans have been described and/or recorded in host species (Figure III).
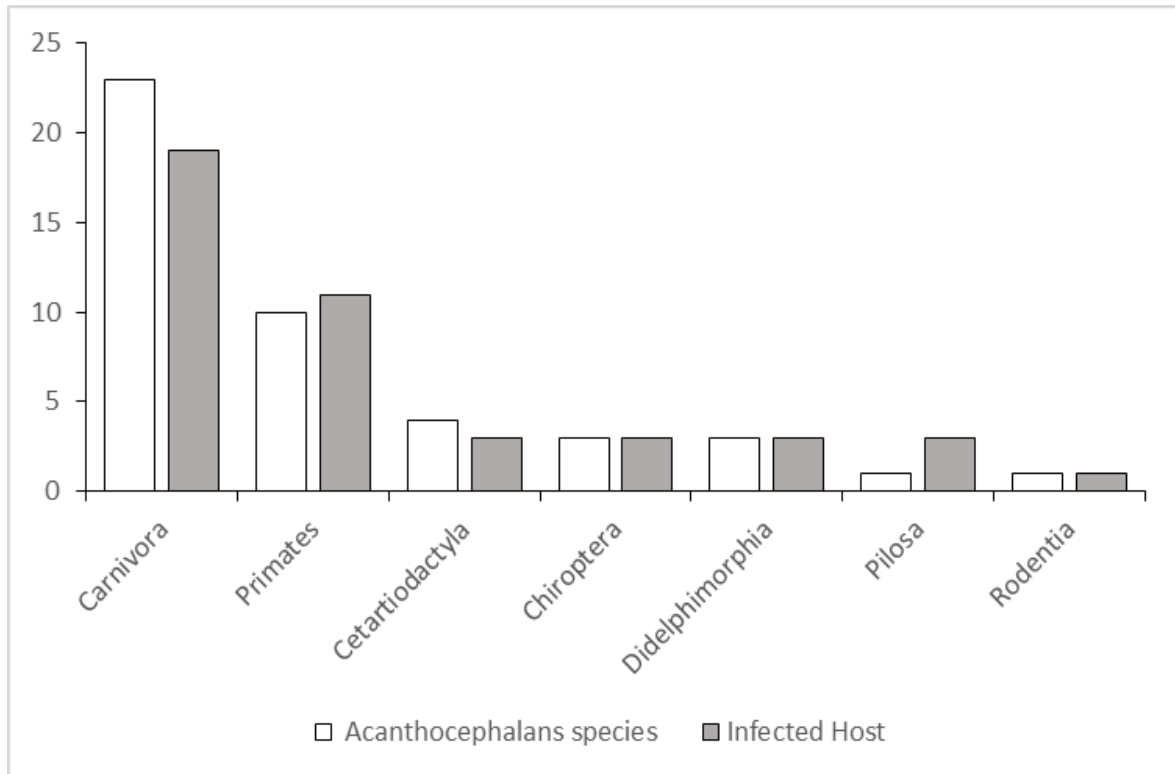
Figure III. Number of acanthocephalan species described in different orders of mammals in Brazil, according to reports available in the literature. Bars in hatched indicate the number of acanthocephalan species and bars in grey indicates the number of mammals infected by acanthocephalans**.**

Even though Brazil has a large diversity of mammal species (about 701), of which 33 are carnivores and 118 primates (Paglia et al., 2012), the number of acanthocephalan species reported in those hosts is still considered low. Recently, Amin (2013) updated the classification of the phylum Acanthocephala and considered 1300 valid species, of which only 3% are species from mammals in Brazil. The description of species found in mammals in Brazil needs to be better detailed because there is little taxonomic information (Travassos, 1915; Travassos, 1917; Machado Filho, 1950; Vieira et al., 2008; Muniz-Pereira et al., 2009). Furthermore, there is a lack of molecular data in public databases.

1.3 Thesis proposal and structure

Parasites are important members of global biodiversity, with helminths being considered a diverse group within metazoan parasites of vertebrates (Mouritsen and Poulin, 2002; Poulin and Morand, 2004). The phylum Acanthocephala has been reported in different host vertebrates and geographic regions in Brazil. However, most of the taxonomic studies need revision of the taxa due to incomplete taxonomic information (Travassos, 1915; Travassos, 1917; Machado Filho, 1950; Vieira et al., 2008; Muniz-Pereira et al., 2009). Molecular and ecological studies are still scarce involving Brazilian acanthocephalans in mammals (Amin et al., 2014, 2016, 2019; Santos et al., 2017). Currently, multiple disciplines are being used together to describe acanthocephalan species, such as morphology, genetics and molecular phylogeny (Amin et al., 2013, 2016, 2019; García-Varela et al., 2005; Hernández-Orts et al., 2017; Li et al., 2017; Malyarchuk et al., 2014). During the field studies carried out by the Laboratory of Biology and Parasitology of Wild Mammal Reservoirs (*Laboratório de Biologia e Parasitologia de Mamíferos Silvetres Reservatórios - FIOCRUZ*) in different regions of Brazil, some specimens of acanthocephalans were collected in the rodent hair-tailed bolo mouse (*Necromys lasiurus*), the carnivore brown-nosed coati (*Nasua nasua*) and the giant anteater (*Myrmecophaga tridactyla*). Therefore, in this study I have described several species of acanthocephalans by integrative taxonomy using morphological and genetic characteristics, and molecular phylogeny. The study also provides ecological information on two acanthocephalan species in carnivores. The thesis is divided into four chapters. The first chapter provides ecological analysis of how biotic and abiotic features influence parasitological parameters of acanthocephalan infection in brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) in the Brazilian Pantanal, as a follow-up study of my master's thesis. In the second chapter, I describe a new acanthocephalan species from brown-nosed coatis with notes on the genus and a key for species identification. In the third chapter, I redescribe a species from the giant anteater adding morphological and molecular data with molecular phylogenetic analysis. Finally, in chapter 4, I describe a new species from the hairy-tailed bolo mouse (*Necromys lasiurus*) in the Cerrado biome, including molecular and phylogenetic data.

2 OBJECTIVES

2.1 General Objective

To carry out the integrative taxonomy of acanthocephalans parasite from mammals of the families Procyonidae, Myrmecophagidae and Cricetidae employing morphological, molecular and ecological traits.

2.2 Specific Objectives

- To determine the ecological factors involved in prevalence and abundance of acanthocephalans infection in brown-nosed coati *Nasua nasua* and crab-eating fox *Cerdocyon thous* by coproparasitological analysis of feces.

- To describe the morphology of acanthocephalans specimens collected in the Brazilian wild mammals as brown-nosed coati (*Nasua nasua*), giant anteater (*Myrmecophaga tridactyla*) and hairy-tailed bolo mouse (*Necromys lasiurus*) by light microscopy (LM) and scanning electron microscopy (SEM);

- To perform molecular analysis of the acanthocephalans using ribosomal molecular partial gene sequences as 28S rRNA, internal transcribed spacer regions (ITS1 and ITS2), and the partinal mitochondrial cytochrome c oxidase subunit I (MT-CO1) gene sequence; and infer the molecular phylogenetic relationship between the species of the present study and the sequences available on public database;

3 CHAPTER 1: VARIATION IN THE PREVALENCE AND ABUNDANCE OF ACANTHOCEPHALANS IN BROWN-NOSED COATIS *NASUA NASUA* AND CRAB-EATING FOXES *CERDOCYON THOUS* IN THE BRAZILIAN PANTANAL

Gomes et al., 2018. Variation in the prevalence and abundance of acanthocephalans in brown-nosed coatis *Nasua nasua* and crab-eating foxes *Cerdocyon thous* in the Brazilian Pantanal. *Brazilian Journal Biology. Ahead of Print.* https://doi.org/10.1590/1519-6984.187881.

Chapter 1

Variation in the prevalence and abundance of acanthocephalans in brown-nosed coatis *Nasua nasua* and crab-eating foxes *Cerdocyon thous* in the Brazilian Pantanal

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Abstract

Host infection by parasites is influenced by an array of factors, including host and environmental features. We investigated the relationship between host sex, body size and age, as well as seasonality on infection patterns by acanthocephalan in coatis (Procyonidae: *Nasua nasua*) and in crab-eating foxes (Canidae: *Cerdocyon thous*) from the Brazilian Pantanal wetlands. Between 2006 and 2009, we collected faecal samples from these hosts and analyzed for the presence of acanthocephalan eggs. Prevalence, abundance and intensity of eggs of acanthocephalans were calculated. Egg abundance was analyzed using generalized linear models (GLM) with a negative binomial distribution and models were compared by Akaike criteria to verify the effect of biotic and abiotic factors. Prevalence of acanthocephalans was higher in the wet season in both host species but did not differ between host sexes; however, adult crab-eating foxes showed higher prevalence of acanthocephalan eggs than juveniles. In contrast, prevalence of acanthocephalan eggs found in coatis was higher in coati juveniles than in adults. Host age, season and maximum temperature were the top predictors of abundance of acanthocephalan eggs in crab-eating foxes whereas season and host sex were predictors of egg abundance in coatis. The importance of seasonality for abundance of acanthocephalan was clear for both host species. The influence of host-related attributes, however, varied by host species, with host gender and host age being important factors associated with prevalence and parasite loads.

Keywords: Acanthocephala, Carnivora, disease ecology, helminth, Pantanal.

Variação na prevalência e na abundância do parasitismo de acantócefalos em dois carnívoros silvestres do Pantanal brasileiro

Resumo

A infecção de hospedeiro por parasitos é influenciada por uma série de fatores, incluindo características do hospedeiro e ambientais. Nós investigamos a relação entre sexo do hospedeiro, tamanho corporal e idade, bem como sazonalidade nos padrões de infecção por acantocéfalos em coatis (Procyonidae: *Nasua nasua*) e em cachorro-do-mato (Canidae: *Cerdocyon thous*) do Pantanal brasileiro e quais fatores explicaram melhor a prevalência e a intensidade desses parasitos. Entre 2006 e 2009, coletamos amostras fecais desses hospedeiros e analisamos a presença de ovos de acantocéfalos. Prevalência, abundância e intensidade de ovos de acantocéfálios foram calculados. A abundância de ovos foi analisada utilizando modelos lineares generalizados (GLM) com distribuição binomial negativa e os modelos foram comparados pelo critério de *Akaike* para verificar o efeito de fatores bióticos e abióticos. A prevalência de acantocéfalos foi maior na estação úmida em ambas as espécies de hospedeiros, mas não diferiu entre os sexos do hospedeiro; no entanto, os cachorros-do-mato adultos apresentaram maior prevalência de ovos de acantocéfalos do que em juvenis. Em contraste, a prevalência de ovos de acantocéfalos encontrados em coatis foi maior em juvenis do que em adultos. A idade do hospedeiro, a estação e a temperatura máxima foram os preditores de abundância de ovos de acantocéfalos em cachorro-do-mato, enquanto a estação e o sexo do hospedeiro foram preditores da abundância dos ovos do parasito em coatis. A importância da sazonalidade para a abundância do acantocéfalo foi clara para ambas as espécies hospedeiras. A influência dos atributos relacionados ao hospedeiro, no entanto, variou entre as espécies de hospedeiros, sendo o sexo e idade do hospedeiro fatores importantes associados à prevalência e às cargas parasitárias.

Palavras-chave: Acanthocephala, Carnívora, ecologia de doença, helminto, Pantanal.

3.1 Introduction

Helminth parasites show a variety of transmission patterns determined by their life cycle characteristics and ecological requirements. As a result, their prevalence and abundance has been correlated with both life history characteristics of the host as well as environmental factors that act on helminth development (Mas-Coma et al., 2008). While such correlations are now well-recognized for many parasitic taxa, the relative importance these biotic and abitoc factors in explaining variability in the timing of infection is often not fully understood.

Seasonal variation in temperature and humidity and host features such as feeding habits, habitat preference, age, gender and body size can regulate the host-parasitism dynamic and are often considered in ecological studies of many parasites (Behnke et al., 2001; Ferrari, 2005; Krasnov et al., 2005; Simões et al., 2014). Such factors can determine the contact rates, and thereby influencing parasite population dynamics, parasite spatial distribution, and the risk of host infection (Bush et al., 2001; Altizer et al., 2006). Among mammals, males tend to have higher abundance, prevalence and parasite species richness than females (Poulin, 1996; Schalk and Forbes, 1997; Soliman et al., 2001; Rossin and Malizia, 2002). These trends have been related to sex-specific host behaviors, as well as distinct androgen levels, body mass differences, and higher levels of physiological stress (Brown et al., 1994; Arneberg et al., 1998; Moore and Wilson, 2002; Morand et al., 2004; Krasnov et al., 2011). Likewise, older hosts may have higher parasite loads due to the more extensive opportunity for exposure to the parasite throughout their lives (Anderson and Gordon, 1982; Anderson and May, 1991; Cooper et al., 2012; Hudson et al., 2002).

Ecological factors associated with parasitism by endoparasites have primarily focused on nematodes of mammals (e.g. Brouat et al., 2007; Simões et al., 2012; Cardoso et al., 2016; Spickett et al., 2017). Few studies have addressed the Phylum Acanthocephala. Acathocephalans are a group of intestinal parasites with wide geographic distribution and approximately 1,300 species (Amin, 2013). Adult parasites attached to the wall of the intestine in the definitive host, causing various pathological conditions such as chronic enteritis with ulcerative lesions (Dunn, 1963; Müller et al., 2010). They typically display a two-host, indirect life cycle involving a variety of arthropods (insects and crustaceans) as intermediate hosts and vertebrates (fish,

amphibians, reptiles, birds and mammals) as definitive hosts (Read, 1974; Crompton and Nickol, 1985).

The ecology of the Acanthocephala has mainly been studied in aquatic arthropods and aquatic vertebrates (Liat and Pike, 1980; Amin, 1984a, 1984b; Sinisalo et al., 2004; Kennedy, 2006; Steinauer et al., 2006; Franceschi et al., 2008; Amin et al., 2008; Caddigan et al., 2014; Amin, 2016), with limited research on the ecology of acanthocephalans of terrestrial mammals (Kennedy, 2006). For example, to our knowledge there have been no ecological studies of acanthocephalans from mammalian wildlife in Brazil. The aim of this study was to examine how biotic and abiotic features influence parasitological parameters of Acanthocephala found in brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) in the Brazilian Pantanal.

The crab-eating fox *Cerdocyon thous* (Linnaeus, 1766) is a monogamous, sexually monomorphic canid with a social structure composed of two to five individuals, usually a breeding pair with pups and sometimes offspring from previous years (Courtenay and Maffei, 2004; Bianchi et al., 2016). In contrast, the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) is a polygynous, sexually dimorphic species in which adult males are larger than females (Olifiers, 2010). Adult females and juvenile form groups of several individuals, but adults' males are typically solitary outside of the reproductive season (Gompper and Decker, 1998; Bianchi et al., 2014). After the breeding season, pregnant females give birth in a nest, usually constructed on a tree, since this species is scansorial (Olifiers et al., 2009). Both species have generalist omnivorous diets, consuming fruits, gastropods, arthropods such as arachnids, insects, myriapods, as well as small vertebrates (Bianchi et al., 2014; Olmos, 1993; Pedó et al., 2006).

Although both coatis and crab-eating foxes have generalist diets (Bianchi et al., 2014) and inhabit similar habitats, their distinct reproductive behavioral and sex-related morphologic features may result in different infection patterns. As a consequence, parasite load is expected to be higher in coati males than females, but not to differ by gender for the monomorphic crab-eating foxes. On the other hand, patterns of parasitism should also vary with abiotic factors in habitats with strong seasonality. For example, the Brazilian Pantanal, where both coatis and crab-eating foxes are sympatric, presents two makedly different seasons, with higher temperature and humidity during the wet season that can favor the life cycle of parasites and their

intermediate hosts (e.g., for acanthocephalans: Kennedy, 2006; Amin, 1980). If abiotic factors are more important than factors intrinsic to the host in mediating the parasite-host dynamic, we expect the two parasite-host dyads to show similar quantitative relationships despite the differing ecologies of the hosts.

3.2 Material and Methods

3.2.1 Study area

The Pantanal biome is the largest wetland in the world and harbors a high density and diversity of vertebrates, particularly mammals (Tomás et al., 2010; Alho et al., 2011; Alho and Sabino, 2011). Field work was conducted at Nhumirim Ranch (18°59'S, 56°39'W), a 4,400 ha research station of the Brazilian Agricultural Research Corporation (Embrapa) in the Nhecolândia subregion of the Pantanal State of Mato Grosso do Sul, Brazil. The study area is characterized by sandy soil with mosaic vegetation of semi-deciduous forest with open grassy areas and seasonally flooded fields (Rodela, 2006). The climate is tropical with two distinct seasons: wet season (October to March) and dry season (April to September).

3.2.2 Capture procedures

From 2006 to 2009 we captured/recaptured *Cerdocyon thous* and *Nasua nasua* which were the subject of a broader research program conducted by Embrapa/Pantanal and the Oswaldo Cruz Foundation (FIOCRUZ-RJ). As part of this research, we collected feacal samples from known individuals for gastro-intestinal parasite diagnosis. Animals were captured every 3 to 4 months using wire box traps (1 m × 0.40 m × 0.50 m) placed in a trapping grid of 7.2 Km2, but traps were also occasionally placed outside the grid. Traps were baited with bacon, set late in the afternoon and checked in the morning. The captured animals were anesthetized, tagged with numbered colored tag (Nasco Rototag®) and/or subcutaneous transponder (AnimalTag®), measured, weighed and sexed. Tooth eruption, condition and wear were also recorded to age individuals (Olifiers et al., 2010). Feacal samples were collected from beneath traps or via fecal loop. After sample collection, the animals were released at their capture sites. The animal capture and handling procedures were approved by the Brazilian Federal Environmental Agency (IBAMA,

first license #183/2005, CGFAU/LIC; last license #11772-2) and by the University of Missouri Animal Care and Use Committee (protocol #4459).

3.2.3 Parasitological procedures

Feces collected from each animal (1-3g) were stored in 15mL of 10% formalin and analyzed in the laboratory using methods for endoparasites diagnostics: flotation in sugar solution (density 1.27), sedimentation and centrifugation with formol-ether (Bowman, 1999). After sedimentation, the pellet was resuspended in 1 mL of 10% formaldehyde and a subsample of 80 µL was placed on a slide for analysis in the light microscope (Monteiro et al., 2007). Slides from the sugar flotation and sedimentation techniques were analyzed at 100x and 400x magnification. Eggs of acanthocephalans were photographed, measured, and compared with the morphology described according to Yamaguti (1963), Schmidt (1972), and Machado Filho (1950). In addition, adults specimens of acanthocephalans were collected from the intestine of three crab-eating foxes and two brown-nosed coatis found dead in the study area. The adults specimens were analysed and described/identified as the *Prosthenorchis cerdocyonis* (Gomes et al, 2015; type species CHIOC 35804 a-c) and *Pachysentis* sp. (deposit pending), respectively. Because co-infection by acanthocephalan species are apparently rare (Kennedy, 2006) and the eggs found in fecal flotation were very similar in size and shape to the eggs obtained from the female acanthocephalans recovered from the dead hosts, we suggest that we are identifying and quantifying *P. cerdocyonis* from crab-eating foxes and *Pachysenti* sp. from coatis. However, since we cannot discard the possibility of co-infection by other (perhaps undescribed) acanthocephalan species parasitizing coatis and crab-eating foxes in the study area, we classified the eggs as belonged to acanthocephalans from the Class Archiacanthocephala, Order Oligacanthorhynchida, Family Oligocanthorhynchidae. The number of acanthocephalan eggs in the faecal samples was divided by the total weight of analyzed feces and used as proxy of parasite abundance. When more than one sample for the same host was obtained in the same excursion (recaptured animals), we calculated the mean number of eggs obtained for the samples analyzed for that period.

3.2.4 Data analyses

We calculated the prevalence as the estimated number of infected hosts divided by the total number of analyzed hosts. Abundance was estimated as the number of eggs per gram of feces found in each individual host and the intensity was the number of eggs per gram of feces found in infected hosts (Bush et al., 1997). Prevalence was compared between sexes, age and seasons using Chi-square tests (α = 0.05) for each host species. Mean intensity and mean abundance were also compared between species using the program Quantitative Parasitology 3.0 (QP3.0; Reiczigel and Rózsa, 2005). Confidence intervals (95% CI) for prevalence were calculated using the Clopper-Pearson interval method, and for mean and median intensity as well as mean abundance by bootstrap tests (n = 2000) using QP 3.0. The level of aggegration of both acanthocephalan species on their respective hosts was quantified by calculating the negative binomial exponent, k (Wilson et al., 2002).

To analyze the effect of biotic (age, sex, body size) and abiotic factors (season, temperature and humidity) on the abundance acanthocephalan eggs (dependent variable) we created generalized linear models (GLM) with negative binomial distributions and log link in SPSS 20, as the data showed a predominantly aggregated distribution for both parasite species (see results). Before creating the models, we checked whether abiotic variables (minimum, maximum and average temperature, relative humidity and precipitation) were correlated (Pearson correlation, α = 0.05). The final factors used to create the models were maximum temperature (MT), relative humidity (RH) and season (dry and wet season). Abiotic data was obtained from the Instituto Nacional de Meteorologia and averaged for 30 days before the date of the fecal sample collection. Host body size (mm) was measured from the tip of the nose to the base of the tail (Olifiers, 2010). Host age was estimated based on morphometric measurements and dental condition following Olifiers et al. (2010), which allowed placement of animals into one of four age categories. We further combined classes due to small sample sizes for some age groups such that all animals were ultimately classified as juveniles ($\leq$ 2 years old) or adults (> 2 years old).

The evaluated models consisted of all possible combinations of the six independent predictors (64 models in total); five additional models having interaction terms were included after investigation of predictor vs. response variable plots revealed possible interaction between these variables. Models were compared using

the Akaike Information Criterion corrected for overdispersion (QAICc) and ranked based on the difference between the best approximating model (model with the lowest QAICc) and all others in the set of candidate models (ΔQAICc). Models with differences within two units of the top model were considered competitive models with empirical support (Burnham and Anderson, 2001). The relative importance of each predictor or interaction of predictors was quantified by calculating relative variable weights, which consists of the summed Akaike weights (QAICc weights) across all the models in which the predictor occurs. Variables weights lower than 0.40 were considered indicative of relatively low variable importance.

3.3 Results

We analyzed 118 fecal samples from 55 crab-eating foxes (24 females and 31 males) and 72 fecal samples from 61 brown-nosed coatis (13 females and 48 males) throughout 10 field excursions (see Table 1 and 2). Prevalence of acanthocephalan eggs did not differ between crab-eating foxes (22.9%; n = 118) and brown-nosed coatis (29.2%; n = 72; Chi-square = 0.936; p = 0.333). Likewise, mean abundance (t-statistic = -0.607; p = 0.556) and mean intensity (t-statistic = -1.903; p = 0.061) did not differ between host species. Egg abundance was similarly aggregated in both hosts (acanthocephalan eggs in crab-eating foxes: k = 0.1031, Figure 1; acanthocephalan eggs in coatis: k = 0.1734, Figure 2).

Table 1. Ecological parameters for *Prosthenorchis cerdocyonis* eggs in crab-eating foxes (*Cerdocyon thous*) sampled in the Brazilian Pantanal from 2006 to 2009.

Categories	N	Prevalence (%)	Mean Intensity	Median Intensity	Mean Abundance
All	118	22.9 % (15.65-31.52)	6.0 (4.78 -7.93)	4.0 (4.0-8.0)	1.37 (0.89-2.04
Females	55	21.8 % (12.46-34.45)	6.0 (4.67-7.92)	5.0 (4.0-8.0)	1.31 (0.67-2.20
Males	63	23.8 % (13.98-36.22)	6.0 (4.20-9.00)	4.0 (2.0-8.0)	1.43 (0.78-2.59)
Adults	70	27.1% (17.19-39.10)	6.84 (5.32-9.32)	7.0 (4.0-8.0)	1.86 (1.13-2.91)
Juveniles	48	16.7% (7.48-30.23)	4.0 (2.88-5.00)	4.0 (2.0-6.0)	0.67 (0.29-1.21)
Dry season	75	17.3% (9.56-27.82)	7.23 (5.15-11.00)	6.0 (3.0-8.0)	1.25 (0.67-2.29)
Wet season	43	32.6% (19.07-48.55)	4.86 (3.57-6.14)	4.0 (2.0-7.0)	1.58 (0.88-2.47)

Numbers between brackets are 95% confidence intervals; N = number of sampled hosts.

Table 2. Ecological parameters for *Pachysentis* eggs in brown-nosed coatis (*Nasua nasua*) sampled in the Brazilian Pantanal from 2006 to 2009.

Categories	N	Prevalence	Mean Intensity	Median Intensity	Mean Abundance
All	72	29.2% (19.04-41.07)	3.81 (2.52-5.86)	2.0 (1.0-4.0)	1.1 (0.64 -1.96)
Females	13	23.1% (5.03-53.82)	2.0 (1.00-2.67)	2.0*	0.46 (0.08-1.15)
Males	59	30.5% (19.18-43.87)	4.06 (2.61-6.44)	2.5 (1.0-4.0)	1.24 (0.68-2.22)
Adults	26	15.4% (4.35-34.87)	6.5 (3.50-10.75)	5.5*	1.0 (0.27-2.54)
Juveniles	46	37.0% (23.20-52.46)	3.18 (2.00-5.71)	2.0 (1.0-3.0)	1.17 (0.63-2.37)
Dry season	26	11.5% (2,44-30,16)	2.0 (1.00-2.67)	2.0*	0.23 (0.04-0.58)
Wet season	46	39.1% (25.08-54.63)	4.11 (2.67-6.33)	2.5(1.0-4.0)	1.61 (0.87-2.76)

Numbers between brackets are 95% confidence intervals; N = number of sampled hosts; *Confidence level cannot be reached because the sample size is small.

Figure 1. Distribution of acanthocephalan egg abundance (eggs/g of feces) in crab-eating foxes (*Cerdocyon thous*) from the Brazilian Pantanal wetlands.
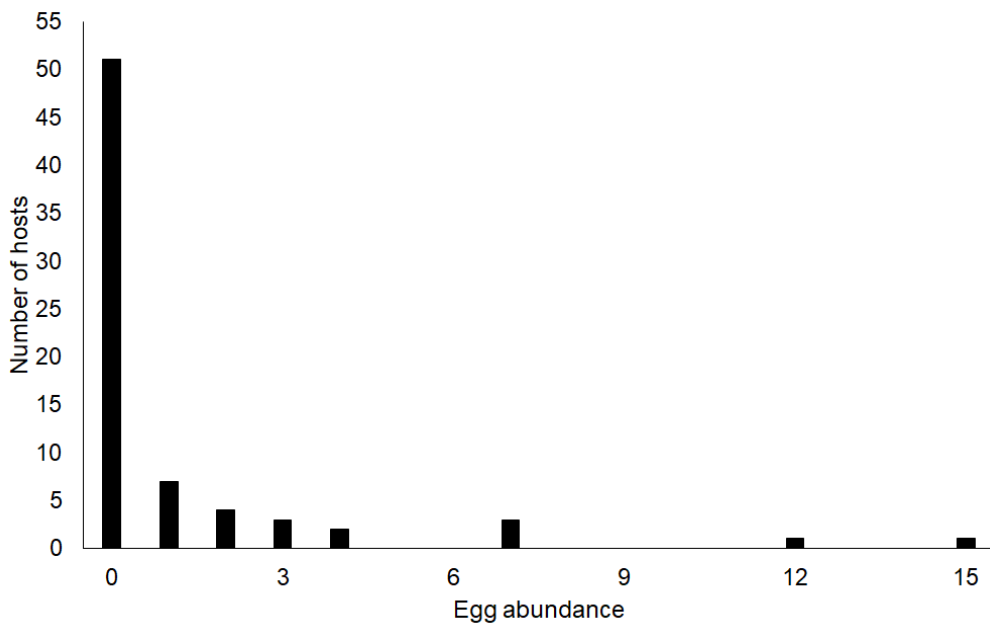
Figure 2. Distribution of acanthocephalan egg abundance (eggs/g feces) in brown-nosed coatis (*Nasua nasua*) from the Brazilian Pantanal wetlands.

3.3.1 Ecological analyses of acanthocephalans in crab-eating foxes (Cerdocyon thous)

Differences in prevalence between host sexes (Chi-square = 0.066, p = 0.797) or age categories were not significant (Chi-square = 1.771; p = 0.183). However, prevalence of eggs tended to be higher during the wet season (32.6%) than in the dry season (17.3%), although the difference was only marginally significant (Chi-square = 3.590, p = 0.058) and 95% CIs of intensity and abundance overlapped.

Four models were supported (ΔQAICc < 2) in the analysis of the abundance acanthocephalan eggs found in crab-eating foxes, but their individual QAICc weights were relatively low (from 0.05 to 0.13; Table 3). The top ranked model supported an interaction of season and age, followed for three models that included maximum temperature either alone or in combination with host age (Table 3). Indeed, the contributions of age (var. weight = 0.75, β = 1.08), maximum temperature (var. weight = 0.56; β = 0.197) and season (var. weight = 0.41; βdry = - 0.43) to variation in abundance of the acanthocephalan eggs in crab-eating foxes were higher than all other variables.

Table 3. Ranking of the best-fitting models describing *P. cerdocyonis* egg abundance in crab-eating foxes (*Cerdocyon thous*) in the Pantanal wetlands, Mato Grosso do Sul, Brazil from 2006 to 2009.

Model	Log(l)/c	QAICc	k	ΔQAICc	QAICc Weight
Season × Host age	-56.30	123.15	5	0.00	0.13
Host age + Max. temperature	-57.76	123.87	4	0.73	0.09
Max. temperature ×Host age	-57.82	123.99	6	0.84	0.09
Max. temperature	-59.46	125.13	3	1.98	0.05

Season = dry and wet seasons; Max. temperature = daily maximum temperature averaged for 30 days before the date of the fecal sample collection. Only models with ΔQAICc ≤ 2 are shown. Akaike Information Criterion corrected for overdispersion (QAICc), Akaike weights (QAICc weights).

3.3.2 Ecological analyzes of acanthocephalan eggs in brown-nosed coatis (Nasua nasua)

Prevalence in coati males and females did not differ (Chi-square = 0.285; p = 0.594), but prevalence was higher in juveniles than in adults (Chi-square = 3.742; p = 0.053). Egg prevalence was over 3 times higher in the wet season than in the dry season (Chi-square = 6.121; p = 0.013) (Table 2). Similarly, measures of intensity and abundance were higher during the wet season and 95% CIs were non-overlapping for the means of both.

Five top models were supported (ΔQAICc < 2) for the abundance of acanthocephalan eggs in coatis, and these models collectively contained five variables: season (var. weight = 0.88, β_{dry} = -1.816), sex (var. weight = 0.46; β_{female} = -1.316), maximum temperature (var. weight = 0.27, β= 0.114), body size (var. weight = 0.26, β = -0.005), and relative humidity (var. weight = 0.24, β = -0.019) occurred in these most-supported models (Table 4). The variable weights for season, which occurred in all five top models, and sex (which occurred in two of the top models) were higher than 0.40, suggestive of strong support.

Table 4. Ranking of the best-fitting models describing abundance of *Pachysentis* sp. eggs in brown-nosed coati (*Nasua nasua*) in the Pantanal wetlands, Mato Grosso do Sul from 2006 to 2009.

Model	Log(l)/c	QAICc	k	ΔQAICc	QAICc Weight
Season	-42.94	92.23	3	0.00	0.13
Season + Host sex	-41.95	92.50	4	0.27	0.11
Season + Humidity	-42.44	93.48	4	1.25	0.07
Season + Body size + Host sex	-41.54	93.99	5	1.76	0.05
Season + Max. temperature	-42.73	94.06	4	1.83	0.05

Season = dry and wet seasons; Max. temperature = daily maximum temperature averaged for 30 days before the date of the fecal sample collection; Humidity = daily averaged for 30 days before the date of the fecal sample collection. Only models with ΔQAICc ≤ 2 are shown. Akaike Information Criterion corrected for overdispersion (QAICc), Akaike weights (QAICc weights).

3.4 Discussion

In this study the overall patterns of prevalence, intensity and abundance were similar for acanthocephalans in both hosts. The samples of the present study were collected in the same study area and both definitive hosts have similar habitats and diets (Olifiers et al., 2010; Bianchi et al., 2014, 2016), which suggests these host species may have similar probabilities of contact with infected intermediate hosts. Although coatis are scansorial and therefore can climb trees, they spend most of their foraging time on the ground (Hirsch, 2009). Prevalence of acanthocephalans in crab-eating foxes was not different between host sexes, and neither host age nor host body size appeared amongst the best-fitting models. Male and female crab-eating foxes are monomorphic in body size and the behavioral, spatial and foraging ecology of males and females are similar (Brady, 1979; MacDonald and Courtenay, 1996; Bianchi et al., 2014; Olifiers et al., 2010). Although some studies have shown that higher androgen levels in males may lead to higher parasite intensity or prevalence (Moore and Wilson, 2002; Muehlenbein and Watts, 2010), this hypothesis does not hold for the acanthcephalans eggs found in crab-eating foxes. It seems that exposure rates to the parasite are similar between sexes and resulted in nearly equivalent parasite profiles for males and females.

In contrast to the crab-eating foxes, adult female and male coatis are behaviourally and spatially segregated during most of the year, with males usually solitary, except in the breeding season (Bianchi et al., 2014). Adult males are also larger than females and engage in agonistic behaviours during the reproductive season (Olifiers, 2010). Consequently, intersexual differences in prevalence, intensity and/or abundance of parasites are expected for this host species, especially during the breeding season, due to different testosterone levels, different consumption rates of food items, and the decreased health condition of breeding season males. Indeed, model analysis for abundance of acanthocephalan eggs in coatis indicated that host sex was an important predictor of infection; male coatis seem to be more affected by parasitism, especially during the breeding season, which may in turn favor higher parasite intensities. Olifiers et al. (2015) found similar results for *Trypanosoma evansi* infection in coatis from the same study site.

Adult crab-eating foxes had more acanthocephalan eggs than juveniles (Table 1). This result is expected, given that adults have more time to accumulate parasites

than younger animals. Older hosts may have been exposed to more parasites during their lifetime, as observed in other studies in which there was a continuous increase in parasite loads with host age or age-associated body size (Anderson and Gordon, 1982; Anderson and May, 1991; Hudson and Dobson, 1995; McCormick and Nickol, 2004). However, coatis showed the opposite pattern, with prevalence (but not intensity) being higher in juveniles than in adults (Table 2). Although such result may be related to acquired immunity with age, it is not clear why this process would occur in coatis but not in crab-eating foxes.

Prevalence of acanthocephalans was higher during the wet season for both host species (Table 1 and 2) and all the best-fitting models had the variable "season" or "maximum temperature" (Table 3 and 4). Thus, acanthocephalans from brown-nosed coatis and crab-eating foxes are likely more available to hosts during the wet season. This availability may reflect an increased abundance in intermediate hosts and changes in exposure rates. Furthermore, model analysis revealed higher parasite abundance for acanthocephalan eggs in coatis feces just after a humid month, while abundance of acanthocephalan eggs in crab-eating foxes was higher just after months with higher maximum temperature. Chubb (1982) and Kennedy (2006) showed seasonal cycles in prevalence and abundance of acanthocephalans that were correlated with temperature. Likewise, Amin et al. (2008) also suggested a seasonal pattern of acanthocephalan infection and showed that prevalence of acantocephalans may increase during the summer in freshwater fishes from Lake Malawi, due to the sexual maturity and breeding activity in the end of winter and early spring. In addition, Amin (1980, 1987b) and Kennedy (2006) analyzed the ecology of intermediate hosts and showed that in warm temperatures, parasite development increases as cystachanths (the infective stage to the definitive host) in the intermediate host; a greater proportion of gravid female worms are found in the definitive host during the summer; and the definitive host consumed more infected intermediate host in the summer, resulting in higher transmission rates.

Although the intermediate hosts of the acanthocephalans studied here are unknown in the Pantanal, arthropods are more abundant in the warmer wet season (Santos-Filho et al., 2008), and both host species may have higher consumption rates of these potential intermediate hosts during the wet season. However, while a primary food item consumed by both host species in the study area were coleopterans, which can be intermediate hosts for acanthocephalans, these were

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more frequently found in fecal samples of these animals in the dry season (Bianchi et al., 2014). The pre-patent period for acanthocephalans (infection of the intermediate hosts by cystacants and the development to adults) and the patent period can vary from weeks to months in acanthocephalans (Nicholas, 1967; Kennedy, 2006). If we consider the pre-patent period of acanthocephalans from mammals as 30 to 100 days (Nicholas, 1967; Crompton and Nickol, 1985), the acanthocephalan eggs would be more abundant in coati and fox feces in the wet season if those hosts were actually infected by mid-late dry season. However, the lack of knowledge regarding the life cycle and intermediate host species for these acanthocephalans precludes fully informed inferences regarding the mechanisms driving seasonal variation in parasite loads.

Overall, while the importance of seasonality for acanthocephalan was clear in both host species, the influence of host-related attributes varied for parasite-host interactions. Nonetheless, both host gender and host age appear to be important factors determining prevalence and parasite intensity of these acanthocephalans. The fact that general patterns of prevalence in the Pantanal did not differ between host species, and were similar for both genders in coatis and crab-eating foxes may indicate that differences in features such as body size and social behavior are relatively less important for predicting infection rates by acanthocephalans when compared to the availability and consumption rates of infected intermediate hosts by definitive hosts. Parasites loads, in turn, may shaped more by features related to host health and immune system function, which are in turn potentially affected by host age and gender.

Despite the study using survey approaches that focus on eggs rather than larval or adult stages, we were able to detect important patterns in acanthocephalan ecology, perhaps due to our relatively large sample sizes. We believe that using egg counts is a potentially powerful tool when sample sizes are large and when it is possible to obtain replicates from the same hosts. Morover, fecal egg counts represent a minimally invasive method for estimating parasite loads (Hämäläinen et al., 2015). The study of parasite dynamics in large animals using egg counts is particularly useful considering that many large host species show decreasing abundance and are already threatened by extinction (IUCN, 2008), which precludes host collection for parasite quantification.

4   CHAPTER 2: A NEW SPECIES OF *PACHYSENTIS* MEYER, 1931 (ACANTHOCEPHALA: OLIGACANTHORHYNCHIDAE) IN THE BROWN-NOSED COATI *NASUA NASUA* (CARNIVORA: PROCYONIDAE) FROM BRAZIL, WITH NOTES ON THE GENUS AND A KEY TO SPECIES

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Chapter 2

A new species of *Pachysentis* Meyer, 1931 (Acanthocephala: Oligacanthorhynchidae) in the brown-nosed coati *Nasua nasua* (Carnivora: Procyonidae) from Brazil, with notes on the genus and a key to species

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Running Title: A new species of *Pachysentis* from Brazil

Abstract

Pachysentis lauroi n. sp*.* (Oligacanthorhynchidae: Acanthocephala) is described from the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (Procyonidae: Carnivora) in the Brazilian Pantanal wetlands of the Mato Grosso do Sul State, Brazil. Specimens were studying using light and scanning electron microscopy. The new species is distinguished from other species of *Pachysentis* by the number of hooks in each longitudinal row (12 rows of 4 hooks, total of 48 hooks), presence of barbs on all hooks, and the organization of the cement glands. Notes on the genus *Pachysentis* Meyer, 1931 and a key to its species are provided. Critical comments on some species with a dubious diagnosis and questionable or missed key taxonomic characteristics are also reviewed. We also discuss the zoogeography of the members of the genus.

Keywords: Acanthocephala, *Pachysentis lauroi* n. sp., key to species, carnivore, MatoGrosso do Sul, Brazil.

4.1 Introduction

Pachysentis Meyer, 1931 comprises 10 species, which have been reported parasitizing mammals in Africa and in American continent (Meyer, 1931, Van Cleave, 1953, Golvan, 1957, Machado Filho, 1950, García-Prieto et al. 2012; Vieira et al., 2008, Corrêa et al., 2016, Muniz-Pereira et al., 2016). Acanthocephalans of wild Brazilian mammals have been studied mainly by Travassos (1915, 1917, 1926, Travassos et al.,1927) and Machado-Filho (1940, 1950), who described six species belonging to *Pachysentis,* five of these being reported in Brazil by Machado-Filho (1950) and Vieira et al. (2008). These species are (1) *Pachysentis gethi* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis gethi* Machado-Filho, 1950] from *Eira barbara* (Linnaeus,1758) (Carnivora, Mustelidae) in Pará and Rio de Janeiro States and from *Galictis cuja* (Molina, 1782) and *G. vittata* (Schreber, 1776) in Rio de Janeiro (Machado-Filho 1950; Vieira et al. 2008; Muniz-Pereira et al. 2016); (2) *Pachysentis procyonis* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis procyonis* Machado-Filho, 1950] from *Procyon cancrivorus* (Cuvier, 1798) (Carnivora, Procyonidae) in Rio de Janeiro State (Machado-Filho, 1950); (3) *Pachysentis rugosus* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis rugosus* Machado-Filho, 1950] from *Sapajus cay* (Illiger, 1815) (Primates, Cebidae) in Rio de Janeiro State; (4) *Pachysentis septemserialis* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis septemserialis* Machado-Filho,1950] from *Saguinus niger* (Hoffmannsegg, 1807) (Primates, Callitrichidae) in the Pará State (Machado-Filho, 1950; Corrêa et al., 2016); (5) *Pachysentis lenti* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis lenti* Machado-Filho, 1950] from *Callithrix geoffroyi* (Humboldt, 1812) (Primates, Callitrichidae) in Espírito Santo State.

The brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (Procyonidae) is a medium-sized carnivore abundant in many regions of South America (Alho et al. 1987; Bianchi et al. 2016), especially in the Pantanal wetlands region (Bianchi et al. 2014; Bianchi et al. 2016). A few species of acanthocephalans have been reported infecting *N. nasua,* including *Oncicola luehei* (Travassos, 1917) Schmidt, 1972 in Pará, São Paulo, Minas Gerais, Mato Grosso, and Mato Grosso do Sul States (Travassos, 1917; Lent and Freitas1938; Machado-Filho 1950; Vieira et al. 2008) and *Neoncicola potosi* (Machado-Filho, 1950) Schmidt, 1972 in Foz de Iguaçú, Paraná State (Moraes, 2016).

In this study, a new species, *Pachysentis lauroi* n. sp. is described using light microscopy and scanning electron microscopy (SEM) from the brown-nosed coati in the Brazilian Pantanal wetlands.

4.2 Material and Methods

Two adult brown-nosed coatis were found between 2007 and 2008 at the Nhumirin Ranch (18°59'S, 56°39'W), a research station of the Brazilian Agricultural Research Corporation (Embrapa/Pantanal) in the Nhecolândia subregion of the Pantanal, Mato Grosso do Sul State in the Brazilian Pantanal wetlands. The animals were collected during a research project investigating the ecology and health of wild carnivores. This research project included an inventory of helminth endoparasites. Acanthocephalan specimens were made available to parasitologists at the Oswaldo Cruz Foundation in Rio de Janeiro (FIOCRUZ/RJ). Animal procedures approved by the Brazilian Federal Environmental Agency (IBAMA, first license #183/2005, CGFAU/LIC; last license #11772-2) were followed.

The animals were necropsied and acanthocephalan specimens were collected from the small intestine of each individual host and stored in AFA (alcohol + formalin + acetic acid) for 24 hours and stored in 70% ethanol. Worms used for microscopical studies were stained with acid (hydrochloric) carmine, dehydrated in a graded ethanol series, cleared in phenol 90% and mounted in Canada balsam (modified from Amato, 1985), examined using an Axion Scope A1 Light Microscope (Zeiss,Göttingen, Germany), and illustrated with the aid of a drawing tube attached a Zeiss standard 20 light microscope (Zeiss, Göttingen, Germany).

Generic identification was based on the taxonomic key proposed by Schmidt (1972) and specific taxonomic descriptions. The description of the new species of *Pachysentis* was based on 11 specimens (six males and five females). Measurements are in millimeters unless otherwise stated. The range was followed by the mean in parentheses. Proboscis hooks were counted in longitudinal alternating rows; hooks were measured in terms of its total length: from basal region of hook to the tip, length of the root, and were measured hook + root (tip of the hook to base of the root). The accepted species of *Pachysentis* deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz - CHIOC (Helminthological Collection of the Oswaldo Cruz Institute), *P.gethi* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC

15680, 17836 a, 17837 b-d, 17838 a-b, 17846, 17852, 38100), *P.rugosus* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 17827, 17828 b-c, 17848), *P.procyonis* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 17847, 17833 a-b, 17854), *P.septemserialis* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 10593, 17812 a-b), *P.lenti* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 14830, 17819 a, 17820 a-c) and species deposited in the Museum für Naturkunde, Berlin, *P.procubens* Meyer, 1931 (No. 2440, 2443, 2474, 6032), *P.ehrenbergi* Meyer,1931 (N°2426, 2432, 6033), *P.canicola* Meyer, 1931 (No.2571) were used for comparison. Specimens of *Pachysentis lauroi* n. sp were deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil, under the number CHIOC no. 38565a (holotype) and 38565b (allotype).

For SEM, the specimens were fixed for one hour at room temperature in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, washed in the same buffer and post-fixed for three hours at room temperature in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer. The material was then dehydrated in ascending ethanol series, critical point dried with CO_2, mounted with silver cello tape on aluminum stubs, and sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LV microscope (JEOL, Akishima, Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

4.3 Results

4.3.1 Description

Order Oligacanthorhynchida Petrochenko, 1956
Family Oligacanthorhynchidae Southwell et Macfie, 1925
Pachysentis lauroi n. sp. (Figs 1-11)
General: With characters of *Pachysentis*as designated by Schmidt (1972). Trunk wider anteriorly. Proboscis subspherical with 12 longitudinal rows of four hooks each, totaling 48 hooks (Figs. 1 and 2). Proboscis hooks similar in size and shape in both sexes. Apical hooks (types I and II) large with posterior curvature, complex manubria and double roots expanding laterally (Fig. 2). Proximal rows with short hooks (types III and IV) and simple discoid roots (Fig. 2). Measurements of length of apical and proximal hooks: length of hook ✕ length of root and [length from proximal extremity to distal extremity in parentheses] in micrometers: (I) 150-229 (182) ✕ 142-203 (170)

[197-207 (249)]; (II) 97-145 (115) × 58-113 (81) [126-184 (153)]; (III) 45-118 (70) × 21-53 (39) [61-129 (91)]; (IV) 26-87 (53) × 18-39 (27) [39-103 (63)]. Hooks with terminal barbs visible by light microscopy in all types of hooks (Figs. 2, 8, 9, 10). Base of proboscis surrounded by lateral papillae with elevated border and central pore (Figs. 1, 6, 7); single apical papilla present with elevated border and salient tip at center (Figs. 6, insert). No marked neck. Proboscis receptacle similar in shape and size in both sexes, with two sub regions measuring 0.87-1.33 (1.16) × 0.43-0.56 (0.47), with cephalic ganglion region (Fig. 1). Lemnisci long, flattened and curved (Fig. 5).

Males (based on six specimens): Trunk6.00-16.61 (9.63) × 1.53-2.53 (1.91) wide anteriorly (Fig. 5). Proboscis 0.51-0.73 (0.64) × 0.68-0.85(0.73) wide. Lemnisci 4.75-6.83 (5.60), reaching middle of trunk (Fig. 5). Reproductive system in posterior 2/3 of trunk. Testes almost equatorial, contiguous, ellipsoid, in tandem (Fig. 5). Anterior testis 0.85-1.76 (1.15) × 0.32-0.62 (0.48); posterior testis 0.90-1.90 (1.27) × 0.48-0.60 (0.55) (Fig. 5). Eight compact uninucleate cement glands, 0.72-1.22 (0.86) × 0.44-0.68 (0.56). Ejaculatory duct 1.10-2.13 (1.42). Copulatory bursa terminal, retracted in all specimens (Fig. 5).

Females (based on five specimens): Trunk 10.79-12.95 (12.07) × 0.53-2.45 (1.62) anteriorly. Proboscis 0.53-0.87 (0.73) × 0.68-0.83 (0.78). Lemnisci 3.30 long in 1 specimen; others masked by eggs. Gonopore subterminal (Fig. 3). Vagina 0.16-0.21 (0.19) long (Figs. 3, 11); uterus 0.61-0.96 (0.80); uterine bell 0.23-0.38 (0.31) × 0.29-0.32 (0.30) (n=2) (Fig. 3). Total reproductive system 1.11-1.34 (1.19) (n=3). Eggs ellipsoidal, with sculptured outer membrane, 0.064-0.082 (0.073) × 0.054-0.036 (0.045) (n=29) (Figs. 4).

Taxonomic Summary

Type host: *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (brown-nosed coati).

Type locality: Nhumirim Ranch (18°85'90S, 56°83'90W), Mato Grosso do Sul State, Brazil.

Site of infection: Small intestine

Etymology: The new species is named in honour of Dr. Lauro Travassos, who contributed greatly to our knowledge of the Brazilian Acanthocephala.
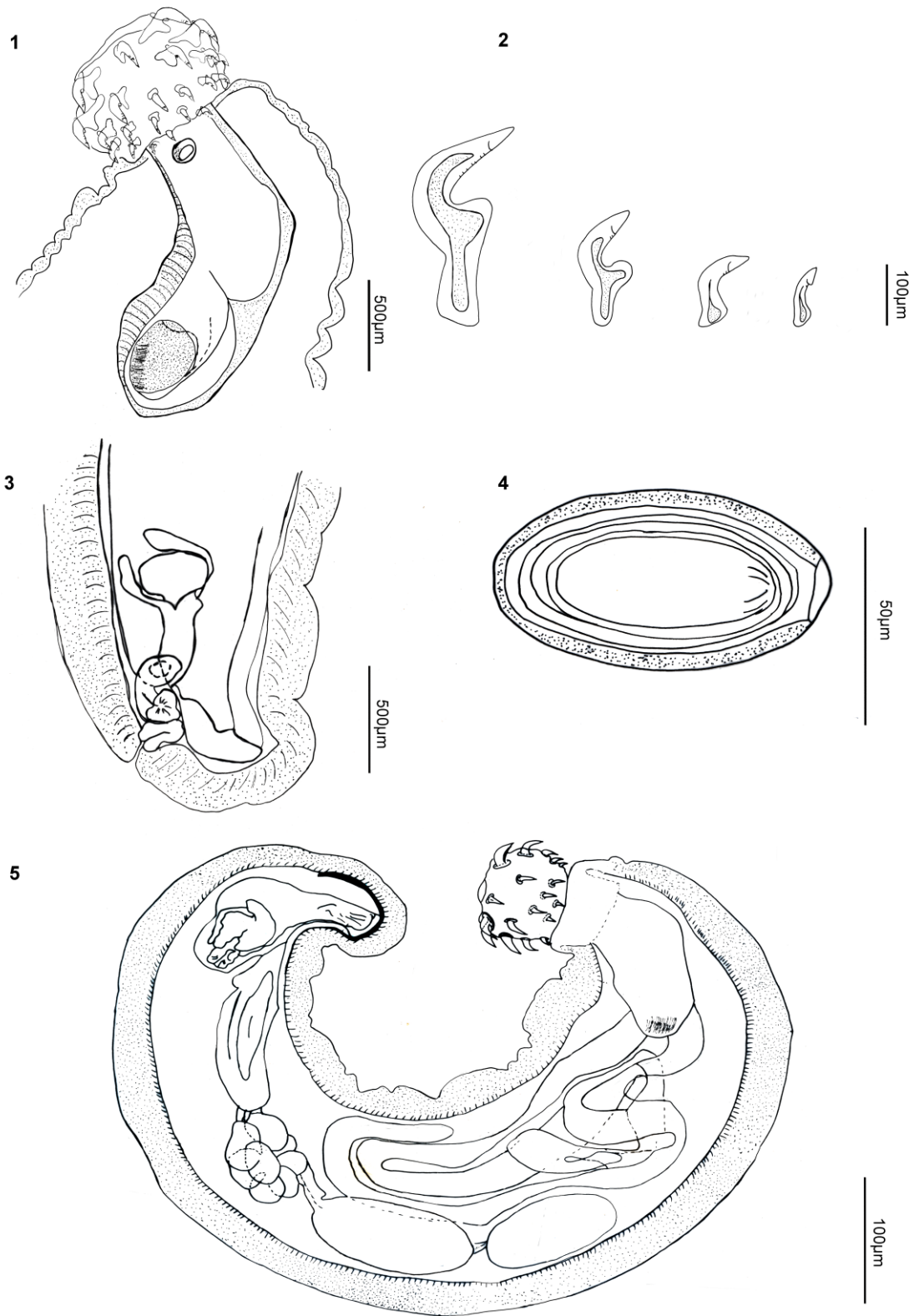
Figure 1-5. Line drawing of *Pachysentis lauroi* n. sp. collected in the intestine of *Nasua nasua* from the Brazilian Pantanal Wetlands, Mato Grasso do Sul State. 1. -globular proboscis with hooks and proboscis receptacle with cephalic ganglion in proximal region; 2. - row with 4 hooks, apical hooks with double root and proximal hooks with simple root; 3. - posterior region of female showing the vagina, uterus and uterine bell; 4. - ellipsoidal egg; 5. -adult male showing two testes, cements glands, ejaculatory ducts and retracted copulatory bursa.
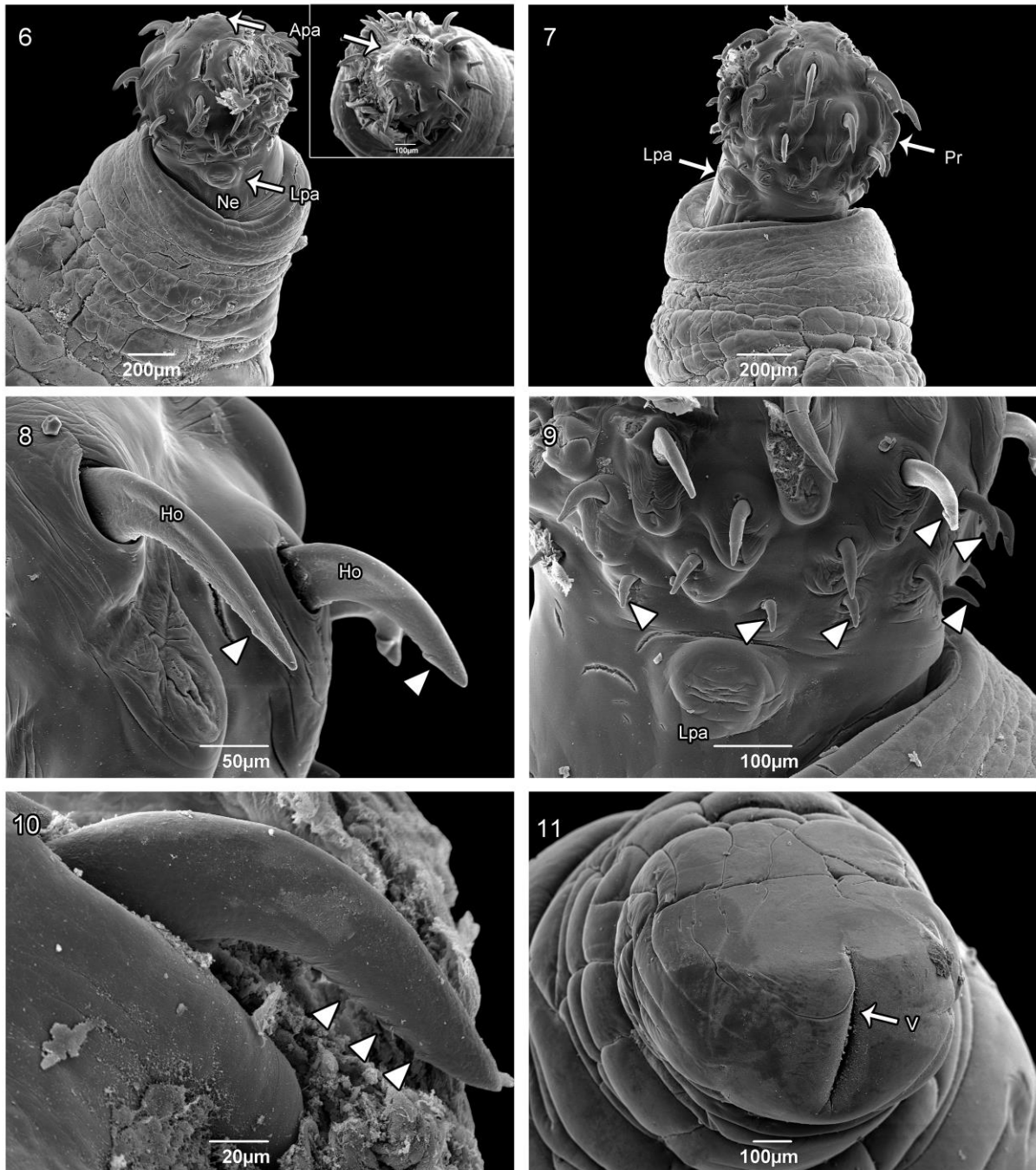
Figure 6-11. Scanning electron micrographs of specimens of *Pachysentis lauroi* n. sp. from *Nasua nasua* in the Brazilian Pantanal Wetlands, Mato Grosso do Sul State. 6 and 7–globular proboscis with lateral papillae and apical papilla; 8 and 9–apical and proximal hooks at base of the proboscis with barbs on the tips of the hooks (arrowhead); 10-detail of the barbs on the tip of the apical hooks (arrowhead); 11-posterior end of female body with subterminal vagina. Lpa, lateral papillae; Apa, apical papilla; Ne, neck; Pr, proboscis; Ho, hook; V, vagina

4.3.2 Remarks

In this study, we identified the specimens obtained from *Nasua nasua* (Linnaeus, 1766) Storr, 1780 as belonging to the Oligacanthorhynchidae and *Pachysentis* due to the presence of a subspherical proboscis, anterior trunk wider than posterior, proboscis with 48 hooks in 12 longitudinal rows of four hooks each using (Schmidt, 1972). In addition, Machado-Filho (1950) considered the number of hooks on the proboscis and the size of the testes as the best characteristics for identifying and distinguishing species of the genus. *Pachysentis lauroi* n. sp. is compared with the other valid species of *Pachysentis* in Table 1 and further distinguished in the dichotomous key presented below.

The status of *Pachysentis septemserialis* Machado-Filho, 1950

The specimens from CHIOC (17812 a-b and 10593) were carefully studied and it was observed that they exhibited some morphological characters not mentioned in the original description. The paratype (permanent slides CHIOC 17812 a-b) was not informative regarding the number of hooks, and a collar was observed at the base of the proboscis, suggesting affiliation with the genus *Prosthenorchis* Travassos, 1915. The female paratype from CHIOC 10593 has 12 longitudinal rows of four hooks with total of 48 hooks, which contradicts the number of the hooks given in the original description (seven rows of seven hooks, total 49 hooks) with no collar at the base of the proboscis (Machado-Filho 1950). Additionally, there is a lack of some information on this species, such as the taxonomic and morphometric characters of adult males. Therefore, we suggest that the specimens designated as *P. septemserialis* (Machado-Filho, 1950) Schmidt, 1972 may be synonymous with *P. lenti* (Machado-Filho, 1950) Schmidt, 1972, as to the number of the hooks, other morphometric characteristics and the fact that both are parasites of primates of the family Callitrichidae. The taxonomy of this species needs to be revised.

The status of *Pachysentis ehrenbergi* Meyer, 1931

Specimens of *Pachysentis ehrenbergi* Meyer, 1931 deposited in the Museum für Naturkunde from *Vulpes vulpes* (No. 2426) and *Naja haje* (No. 2432, 6033) were also examined. Specimens from both hosts had barbs on the tip of all hooks, which was not mentioned by Meyer (1931) in the original description. Other morphological

characteristics, such as the number of hooks, short neck, the presence and size of nuclei in the leminisci and the reproductive organs agree with the original description.

Pachysentis lauroi n. sp. distinguished from the other species of *Pachysentis* by a combination of morphological characters, including the number of the hooks in each longitudinal row, the presence of barbs on the hooks and the arrangement of the cement glands (Table 1). The following key and Table 1 do not include *P. septemserialis,* because of its uncertain taxonomic status, but enable the new taxon to be distinguished from the other nine recognized species of the genus.

1. Proboscis with 12 longitudinal rows, alternating or not, of 3 to 4 hooks --2
-   Proboscis with 12 alternating longitudinal rows of 7 to 9 hooks --- 9
2. Proboscis with a total of 42 to 48 hooks --------------------------------------- 3
-   Proboscis with a total of 72 hooks --- *P. canicola* Meyer, 1931
3. Proboscis with a total of 42 hooks --- 4
-   Proboscis with a total of 48 hooks --- 5
4. Cement glands in pairs --- 6
-   Cement glands clustered -- 7
5. Hooks with visible barbs ("arrow-shaped hook tip") ------------------------------- 8
-   Hooks without barbs --- *P. lenti* (Machado-Filho, 1950) Schmidt, 1972
6. Parasite of carnivores in Africa --- *P. angolensis* (Golvan, 1957) Schmidt, 1972
-   Parasite of carnivores in the Americas --- *P. gethi* (Machado-Filho, 1950) Schmidt, 1972
7. Very short lemnisci not reaching anterior testis. Parasites of carnivores --- *P. procyonis* (Machado-Filho, 1950) Schmidt, 1972
-   Leminisci reaching anterior testis. Parasites of primates --- *P. rugosus* (Machado-Filho, 1950) Schmidt, 1972
8. Cement glands in pairs --- *P. dollfusi* (Machado-Filho, 1950) Schmidt, 1972
-   Cement glands in clusters --- *P. lauroi* n. sp.
9. Proboscis 0.55 mm wide, with a total of 90 hooks without barbs --- *P. procumbens* Meyer, 1931
-   Proboscis 0.8-0.9 mm wide, with a total of 102 hooks with barbs -- *P. ehenbergi* Meyer, 1931

Pachysentis lauroi n. sp. is further distinguished from *P. angolesis, P. canicola, P. procumbens, P. ehrenbergi, P. gethi, P. procyonis* and *P. rugosus* by the number of hooks in each row, with 12 longitudinal rows of four hooks each, totaling 48 hooks (Table 1). Our specimens were similar to *P. lenti* and *P. dollfusi* in the number of hooks (48) on the proboscis. The new species can, however, be distinguished from *P. lenti* by having barbs on all hooks and from *P. dollfusi* by the

organization of the cement glands (in cluster *vs* in uniform pairs), the size of trunk and the definitive host (Table 1). In addition, when Machado Filho (1950) described *P. dollfusi*, he indicated that this acanthocephalan infected a zoo animal in Brazil and that is native of Madagascar. Golvan (1994), however, warned that the origin of this species might not have been Madagascar. Nevertheless, it is not known whether the species originates in Brazil or Madagascar.

Table 1. Morphometric comparison of species of the genus *Pachysentis* (measurements in mm)

Characteristcs/Species	P. angolensis		P.canicola (type species)		P.procumbens (juvenile)		P.ehrenbergi		P.rugosus		P.procyonis	
Author	Golvan, 1957		Meyer, 1931		Meyer, 1931		Meyer, 1931		(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972	
type-host	*Canis adustus*		Dog (Meyer, 1931)		*Vulpes vulpes*		*Vulpes vulpes; Naja haje*		*Sapajus cay*		*Procyon cancrivorus*	
type-locality	Angola, Africa		Brazil, South America		Argo, Egito, Africa		Egito, Africa		Rio de janeiro, Brazil		Rio de janeiro, Brazil	
Trunk	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	17-23 X 3.5-4	34-48 X 4.8-5.5	15-28 X 4-8	20-26 X 5-11	6 X 1.25	6 X 1.25	25 X 4	26-29 X 6	25 X 3.5	32 X 3	20-30 X 2-3	25-35 X 2-3
Proboscis	0.55-0.63 X 0.70-0.82		0.57-0.80 X 0.57-0.85		0.55 X 0.55		0.8 X 0.9		0.564 X 0.694		0.697 X 0.716	
Total number of hooks	42		72		90		102		42		42	
Hooks per row	6 x 4 + 6 x 3		6 x 4 + 12 x 4*		6 x 7 + 6 x 8		6 x 9 + 6 x 8		6 x 4 + 6 x 3		6 x 4 + 6 x 3	
Barbs in hooks	no barbs		no barbs		no barbs		barbs		no barbs		no barbs	
Proboscis receptacle	1.5		2		1.2		1.3		1.24 X 0.481		1.37 X 0.531	
Leminisci	5.8-6		7		-		7 X 0.8		4.64		3.64	
Anterior testis	2-3 X 0.9	-	2	-	-	-	3	-	1.57 X 0.697	-	3.01 X 1.24	-
Posterior testis	2-4.3 X 1.0	-	2	-	-	-	3	-	1.69 X 0.664	-	3.15 X 1.07	-
Dimension of group of cement gland	3	-	3	-	-	-	7	-	2.02	-	3.56	-
Ejaculatory duct length	2.3	-	-	-	-	-	-	-	1.68	-	3.53	-
uterine bell	-	-	-	3. 15 - 8.15	-	-	-	-	-	5.86	-	4.64
eggs	-	0.09 X 0.043	-	0.07 x 0.045	-	-	-	0.07 X 0.05	-	-	-	0.071 X 0.042

Table 1. Morphometric comparison of species of the genus *Pachysentis* (measurements in mm) (continued)

Characteristcs/Species	P.gethi		P.lenti		P.dollfusi		Pachysentis louroi n. sp. (present study)	
Author	(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972		present study	
type-host	*Eira barbara*		*Callithrix geoffroyi*		*Eulemur fulvus* (syn. *Lemur fulvus*)		*Nasua nasua*	
type-locality	Pará and Rio de Janeiro, Brazil		Espirito Santo, Brazil		Madagascar, Africa		Mato Grosso do Sul, Brazil	
Trunk	Male	Female	Male	Female	Male	Female	Male	Female
	10-15 X 1.0-2.5	15-25 X 1.5-3	15-20 X 1.0-2.5	20-25 X 2-2.5	50 X 4	50 x 4	9.63 X 1.91	12.07 X 1.62
Proboscis	0.583 X 0.794		0.63 X 0.664		-		0.68 X 0.76	
Total number of hooks	42		48		48		48	
Hooks per longitudinal row	6 x 4 + 6 x 3		6 x 4 + 6 x 4		6 x 4 + 6 x 4		6 x 4 + 6 x 4	
Barbs in hooks	no barbs		no barbs		barbs		barbs	
Proboscis receptacle	1.07 X 0.498		1.32		-		1.16 X 0.47	
Leminisci	3.48		3.15		4.3-6.6		4.45	
Anterior testis	1.40 X 0.581	-	1.76 X 0.51	-	-	-	1.15 X 0.48	-
Posterior testis	1.40 X 0.581	-	1.82 X 0.547	-	-	-	1.27 X 0.55	-
Dimension of group of cement gland	1.54	-	2.98	-	-	-	0.86 X 0.56	-
Ejaculatory duct length	4.64	-	-	-	-	-	1.42	-
uterine bell	-	5.56	-	1.41	-	-	-	1.19
eggs	-	0.084 X 0.054	-	-	-	0.08 X 0.05	-	0.073 X 0.045

4.4 Discussion

Meyer (1931) proposed *Pachysentis* with the type species *P.canicola* Meyer, 1931 from a domestic dog in Brazil. The same species was found infecting a gray fox *Urocyon cinereoargenteus* (Schreber, 1775) (Carnivora: Canidae) in the United States (Buechner, 1944). Two additional species, *P. ehrenbergi* Meyer, 1931 and *P. procumbens* Meyer, 1931, were described from *Vulpes vulpes* (Linnaeus, 1758) in Egypt (Meyer, 1931; Van Cleave, 1953), suggesting that species from this genus are parasites of carnivores (Order Carnivora).

Van Cleave (1953) also studied acanthocephalan parasites from North American mammals and recorded *P. canicola* in the gray fox and the skunks *Mephitis mephitis mesomelas* (Lichtenstein, 1832), *Conepatus leuconotus* (Lichtenstein, 1832) and *Spilogale gracilis leucoparia* (Merriam, 1890), and recognized the three previous species of the genus. Yamaguti (1963) revised the classification of the Acanthocephala and considered their geographic distributions, revised the diagnosis of the genus *Pachysentis* and followed the classification of Meyer (1931) and Van Cleave (1953) with three species in the genus.

Schmidt (1972) revised the family Oligacanthorhynchidae and transferred six species of *Prosthenorchis* Travassos, 1915 to the genus *Pachysentis*, i.e. *P. dollfusi*, *P. gethi*, *P. lenti*, *P. procyonis*, *P. rugosus*, *P. septemserialis* and *P. angolensis* [syn. *Oncicola angolensis* Golvan, 1957]. *Pachysentis* Meyer, 1931 then included a total of 10 species based on morphological features, such as: an anterior trunk wider than the posterior trunk; the absence of a festooned collar; a globular proboscis with 12 longitudinal rows of 3 to 12 hooks, totaling 42 to 102 hooks; larger anterior hooks with complex manubria and roots, as well as rootless posterior hooks; tips of the hooks with or without barbs; long and flattened lemnisci in arranged a band; testes in tandem in the mid-trunk; eight compacted cement glands; and oval eggs with sculptured outer membranes (Yamaguti, 1963; Schmidt, 1972).

According to this classification, the type hosts for species of *Pachysentis* are primates and carnivores with geographic distributions restricted to Africa and North, Central and South America (Meyer, 1931, Van Cleave, 1953, Golvan, 1957, Machado Filho, 1950, García-Prieto et al., 2012; Vieira et al., 2008, Corrêa et al., 2016; Muniz-Pereira et al., 2016). In the revisions by Golvan (1994) and Amin (2013),

the authors updated the classification of the Acanthocephala and considered *Pachysentis* as including 10 valid species described by Meyer (1931), Golvan (1957) and Machado Filho (1950). Therefore, the member species are *P. canicola, P. ehrenbergi, P. procumbens*, *P. angolensis*, *P. dollfusi*, *P. gethi*, *P. lenti*, *P. procyonis*, *P. rugosus* and *P. septemserialis*.

Our study provides details of *Pachysentis lauroi n. sp.* such as reproductive organs of females and males, as well as detail by scanning electron microscopy showing the presence of barbs on hooks in the proboscis, and the apical and lateral papillae-like structure on the proboscis. Furthermore, we are adding new information of morphology of two species, *P. septemserialis* and *Pachysentis ehrenbergi* and their status in the genus. These morphological features help to identify the new species and contributes to the taxonomy of this acanthocephalan genus. Finally, the present study also reports the definitive host – the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 in a new geographical area, which enlarges the geographic distribution of the genus.

Acknowledgements

5 CHAPTER 3: NEW MORPHOLOGICAL AND GENETIC DATA OF *GIGANTORHYNCHUS ECHINODISCUS* (DIESING, 1851) (ACANTHOCEPHALA: ARCHIACANTHOCEPHALA) IN THE GIANT ANTEATER *MYRMECOPHAGA TRIDACTYLA* LINNAEUS, 1758 (PILOSA: MYRMECOPHAGIDAE)

Ana Paula Nascimento Gomes, Clarice Silva Cesário, Natalie Olifiers, Rita de Cassia Bianchi, Arnaldo Maldonado Jr, Roberto do Val Vilela. New morphological and genetic data of *Gigantorhynchus echinodiscus* (Diesing, 1851) (Acanthocephala: Archiacanthocephala) in the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 (Pilosa: Myrmecophagidae). <u>Submitted to International Journal for Parasitology: Parasites and Wildlife.</u>

New morphological and genetic data of *Gigantorhynchus echinodiscus* (Diesing, 1851) (Acanthocephala: Archiacanthocephala) in the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 (Pilosa: Myrmecophagidae)

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Abstract

Gigantorhynchus echinodischus (Diesing, 1851) is a parasite of anteaters in South America. Although described by Diesing, 1851, there is still a lack of taxonomic and phylogenetic information regarding this species. In the present study, we redescribed G. echinodiscus collected from a giant anteater, Myrmecophaga tridactyla Linnaeus, 1758, from Brazilian Cerrado (Savannah) in the state of São Paulo by light and scanning electron microscopy. In addition, phylogenies were inferred from partial DNA gene sequence of the nuclear large subunit ribosomal RNA gene (28S rRNA). We provided details of the proboscis with a crown having 18 large hooks and numerous small hooks, a lateral papilla at the base of the proboscis, a ringed pseudo-segmented body, large testes, cemented glands in pairs, and a non-segmented region in the posterior end of the body, which contributed to the diagnostic of the species. Molecular phylogenetic analysis recovered G. echinodiscus forming a well-supported monophyletic group with Mediorhynchus sp., which was congruent with morphological studies that allocate both genera within the family Gigantorhynchidae. In conclusion, the present work added new morphological and molecular information, emphasizing the importance of adopting integrative taxonomic approaches in studies on Acanthocephala.

Keywords	Gigantorhynchidae; Integrative taxonomy; Phylogenetic systematics; 28S rRNA; Cerrado
Taxonomy	Parasitology, Helminthology
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ABSTRACT

Gigantorhynchus echinodiscus (Diesing, 1851) is a parasite of anteaters in South America. Although described by Diesing in 1851, there is still a lack of taxonomic and phylogenetic information regarding this species. In the present study, we redescribe *G. echinodiscus* collected from a giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758, from the Brazilian Cerrado (Savannah) in the state of São Paulo by light and scanning electron microscopy. In addition, phylogenies were inferred from partial DNA gene sequence of the nuclear large subunit ribosomal RNA gene (28S rRNA). We provide details of the proboscis with a crown having 18 large hooks and numerous small hooks, a lateral papilla at the base of the proboscis, a ringed pseudo-segmented body, large testes, cemented glands in pairs, and a non-segmented region in the posterior end of the body, which contributed to the diagnosis of the species. Molecular phylogenetic analysis recovered *G. echinodiscus* forming a well-supported monophyletic group with *Mediorhynchus* sp., which was congruent with morphological studies that allocate both genera within the family Gigantorhynchidae. In conclusion, the present work adds new morphological and molecular information, emphasizing the importance of adopting integrative taxonomic approaches in studies of Acanthocephala.

Keywords: Gigantorhynchidae; Integrative taxonomy; Phylogenetic systematics; 28S rRNA; Cerrado

5.1 Introduction

The family Gigantorhynchidae Hamman, 1892 is the unique family at the order Gigantorhynchida Southwell and Macfie, 1925 and contains two genera: *Mediorhynchus* Van Cleave, 1916 and *Gigantorhynchus* Hamman, 1892 (Amin, 2013). The genus *Gigantorhynchus* Hamann, 1892 was validated by Yamaguti (1963) and Amin (1985, 2013), and comprises six valid species: *Gigantorhynchus echinodiscus* (Diesing, 1851) (type species) [syn. *Echinorhynchus echinodiscus* Diesing, 1851], *G. lopezneyrai* Diaz-Ungria, 1958, *G. lutzi* Machado Filho, 1941, *G. ortizi* Sarmiento, 1954, *G. ungriai* Antonio, 1958 parasitizing marsupials and anteaters in South America (Yamaguti, 1963; Amin, 1985, 2013); and *G. pesteri* Tadros, 1966 parasitizing baboom in Africa (Tadros, 1966; Amin, 2013). Particularly, *G. echinosdiscus* is distributed over the Neotropical region and have been reported parasitizing anteaters in Brazil (Travassos, 1917; Machado Filho, 1941), Venezuela (Díaz-Ungria, 1958), Panamá (Dunn, 1934), and Trinidad Island (Camerón, 1939) (Table 1).

In Brazil, two species have been reported, *G. lutzi* Machado Filho, 1941 from the bare-tailed woolly opossum *Caluromys philander* Linnaeus, 1758 (Machado Fillho, 1941) and *G. echinodiscus* infecting anteaters, as the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758; the collaret anteater *Tamandua tetradactyla* (Linnaeus, 1758); and the silk anteater *Cyclopes didactylus* (Linnaeus, 1758) (Travassos, 1917; Strong et al., 1926; Machado Filho, 1941) (Table 1). In addition, eggs of *G. echinodiscus* have been recorded in coprolites of *T. tetradactyla* and *M. tridactyla* from an archaeological site in Brazil (Ferreira et al., 1989).

Currently records of *Gigantorhynchus* species are based on morphological data (Travassos, 1917; Machado Filho, 1941; Sarmiento, 1954; Antonio, 1958; Díaz-Ungría, 1958, Tadros, 1966) and genetic data is not available to the genus *Gigantorhynchus* in public databases.

Lately, the nuclear large subunit ribosomal gene (28S rRNA) have been used as molecular marker for phylogenetic inferences on acanthocephalans. For example, to elucidate the relationships amongst the four classes within the phylum Acanthocephala, to solve taxonomic problems at the family level, and to investigate inter and intraspecific genetic variation within acanthocephalan species (García-Varela and Nadler, 2005; García-Varela et al. 2011, Braicovich et al., 2014; García-

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Varela and Pérez-Ponce de León, 2015; Pinacho-Pinacho et al., 2015; Wayland et al., 2015). Therefore, phylogenetic evidence based on 28S rRNA gene may be helpful, integrating and complementing conventional taxonomic studies for different taxa.

In the present study, we redescribed *Gigantorhynchus echinodiscus* by light and scanning electron microscopy (SEM) and contributed with new molecular data and phylogenetic approach of the family Gigantorhynchidae.

Table 1. Reports and geographic distribution of *Gigantorhynchus echinodiscus* in mammals of South America.

Species of host	Family of host	Locality	Author
Cyclopes didactylus	Cyclopedidae	Brazil	Travassos, 1917
Myrmecophaga tridactyla		São Paulo, Brazil	Travassos, 1917
		Brazil	Diesing, 1851; Haman, 1892
		Rio de Janeiro and São Paulo, Brazil	Travassos, 1917
	Myrmecophagidae	Amazon, Brazil	Strong et al., 1926
		Panama City, Panama	Dunn, 1934
Tamandua tetradactyla		Trinidad Island	Camerón, 1939
		Pará, Brazil	Machado Filho, 1941
		Atures, Venezuela	Díaz-Ungria, 1958
		Brazil	Diesing, 1851; Haman, 1892

5.2 Material and Methods

5.2.1 Field study and recovery of acanthocephalan specimens

The giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 was subject of an ecological research program conducted by the São Paulo State University-UNESP/Jaboticabal (*Universidade Estadual Paulisa* - UNESP/Jaboticabal) and the Institute of Research and Conservation of Anteaters in Brazil (*Instituto de Pesquisa e Conservação de Tamanduás no Brasil - Projeto Tamanduá*), aiming to monitor movement and space use by giant anteaters using GPS devices. The study was conducted in Santa Bárbara Ecological Station (*Estação Ecológica de Santa Bárbara* – ECc Santa Bárbara, 22°48'59″S, 49°14'12″W) located in the municipality of Águas de Santa Bárbara, state of São Paulo, Southeastern Brazil. The ECc Santa Bárbara encompasses 2,712 ha of isolated and protected Cerrado remnant in the state of São Paulo and is characterized by a mosaic vegetation of Cerrado *sensu lato*, gallery forest, patches of semideciduous forest, and plantation of exotic *Pinus* and *Eucalyptus* species (Mello and Durigan, 2011).

Anteaters were captured and sedated for biometric measurements, sample collection, and GPS placement (Bertassoni et al, 2017) (collection permits COTEC 429/2014 D23/2013 PGH and SISBIO 38326-5). Two giant anteaters were necropsied revealed presence of parasites in the intestine. After necropsy, the digestive tract was analyzed and helminths were collected from the small intestine, stored in 70% ethanol, and donated to the Laboratory of Biology and Parasitology of Wild Reservoir Mammals (*Laboratório de Biologia e Parasitologia de Mamíferos Silvetres Reservatórios* - LABPMR). At the LABPRM, the acanthocephalan specimens used for morphological characterization were stained with acid carmine, destained in a solution of 2% hydrochloric acid (HCl) and 70% ethanol, dehydrated in a graded alcohol series (70 to 100%), clarified in 90% phenol, whole-mounted as definitive slide in Canada balsam (modified from Amato, 1985), and analyzed using an Axion Scope A1 Light Microscope (Zeiss, Göttingen, Germany). Drawings were made with the aid of camera lucida attached to a Nikonlight microscope Model Eclipse E200MVR (Nikon Corporation, Tokyo, Japan). Measurements were in millimeters unless otherwise stated, range followed by mean within parentheses. The length of proboscis included the neck, with small hooks, plus the crown of hooks (praesoma). We made three length measurements of the hooks with double root:

from the tip of the hook to the root, total length of the hook; and total length of the root. Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (*Coleção Helmintológica do Instituto Oswaldo Cruz* - CHIOC), Rio de Janeiro, Brazil under the number CHIOC n° 38580.

For scanning electron microscopy (SEM) the specimens previously fixed in 70% ethanol were dehydrated in ascending ethanol series (80%, 90%, 100%), dried by the critical point method with CO_2, mounted with silver cellotape on aluminum stubs, and sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LVmicroscope (JEOL, Akishima, Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute (Plataforma de Microscopia Eletrônica Rudolf Barth/IOC- FIOCRUZ).

5.2.2 *Molecular analyses*

For gene sequence studies, specimens preserved in 70 % ethanol were washed in ultrapure water for 24 hours at room temperature. Total genomic DNA was isolated using the QIAamp DNA mini Kit according to the manufacturer's protocol (Qiagen, Venlo, The Netherlands). DNA amplifications by polymerase chain reaction (PCR) were conducted for the partial nuclear large subunit ribosomal RNA gene (28S rRNA) using the primers C1 5′-ACCCGCTGAATTTAAGCAT-3′ and D2 5′-TGGTCCGTGTTTCAAGAC-3′ (Hassouna et al., 1984 - modified from Chisholm et al., 2001). PCR amplifications were performed using Promega PCR Master Mix (Promega Corporation, Madison, Wisconsin, USA). Reactions were 25 μL following the manufacturer's protocol. The thermal-cycling profile was programmed on a thermocycler Eppendorf Mastercycler Epsystem (Eppendorf, Hamburg, Germany) with an initial denaturation step of 95 °C/ 2 min; followed by 40 cycles of 94 °C/ 60 s, 55 °C/ 60 s, and 72 °C/ 60 s; a final extension at 72 °C/ 5 min; and a cool down to 4°C. PCR products were analyzed after electrophoresis on 1.5% agarose gel using GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) by visualizing on UV transilluminator. Successful amplifications were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Sequencing reactions using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) were performed using the same primers mentioned above in a Gene Amp (Applied Biosystems) thermocycler and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems). Both procedures and cycle-sequenced products

precipitations were conducted at the subunit RPT01A – DNA sequencing platform of the Oswaldo Cruz Institute PDTIS/FIOCRUZ.

Chromatograms were initially assembled into contigs, and manually edited for ambiguities using the software package Geneious 9.1.8 (http://www.geneious.com; Kearse et al., 2012). For assessment of phylogenetic relationships of *G. echinodiscus* sequence, we built a matrix with sequences of representatives of the class Archiacanthocephala retrieved from GenBank. Three families, representing three different orders of archiacanthocephalans, were present in our dataset: Oligacanthorhynchidae represented by sequences of the genera *Oligacanthorhynchus* Travassos, 1915, *Macracanthorhynchus* Travassos, 1917, and *Oncicola* Travassos, 1916; Moniliformidae represented by sequences of the genus *Moniliformis* Travassos, 1915; and Gigantorhynchidae represented by a sequence of the genus *Mediorhynchus* Van Cleave, 1916 and our sequence of *Gigantorhynchus* Hamann, 1892. All of these genera infect mammals and *Mediorhynchus* may infect birds, as well. As outgroup we used two genera of the class Palaeacanthocephala (*Acanthocephalus* Koelreuther, 1771 and *Plagiorhynchus* Lühe, 1911) and two genera of the class Eoacanthocephala (*Neoechinorhynchus* Stiles et Hassall, 1905 and *Floridosentis* Ward, 1953) (Table 2).

We aligned all sequences using the Program MAFFT under default parameters in the Geneious package, followed by manual edition of the sequences, removing the non-complementary regions. The sequences were realigned using the Geneious alignment algorithm using as settings global alignment with free end gaps, cost matrix of transition/transversion (5.0/1.0), and same penalty value of six for both gap opening and extension. The resulting aligned matrix was manually trimmed of poorly aligned regions using the Mesquite 3.51 software package (Maddison and Maddison, 2018).

As assessment of the quality of the data, we tested for the presence of phylogenetic signal the Permutation Test Probability - PTP and the G1 tests in the program PAUP 4.0a164 (Swofford, 2003); and for the presence of substitution saturation using the Xia test (Xia et al., 2003, Xia and Lemey, 2009) with analysis performed on fully resolved sites only and a graphic of transitions and transversions versus JC69 model genetic distances (Jukes and Cantor, 1969) in DAMBE 7.0.35 (Xia, X., 2017).

Phylogenetic relationships based on partial 28S rRNA gene sequences were inferred using Maximum Parsimony (MP), maximum-likelihood (ML), and Bayesian Inference (BI) methods. MP was carried out using PAUP 4.0a164 (Swofford, 2003) with tree heuristic search using starting trees via stepwise addition, with 100 random sequence addition replicates, holding 10 trees at each step, and tree bisection and reconnection (TBR) branch-swapping algorithm. Node supports in MP were assessed by non-parametric bootstrap percentages (MP-BP) after 10,000 pseudoreplications. ML was carried out using PhyML 3.0 (Guidon et al., 2010) with tree heuristic search using subtree pruning and regrafting (SPR), with 10 random starting trees, with model selection by the SMS algorithm (Smart Model Selection) (Lefort et al., 2017) under the Akaike information criterion (AIC). Node supports in ML were assessed by approximate likelihood-ratio test (aLRT) for branches (Anisimova and Gascuel, 2006) and by non-parametric bootstrap percentages (ML-BP) after 1,000 pseudo-replications. BI was carried out using MrBayes version 3.2.6 (Ronquist et al., 2012) on the CIPRES Science Gateway platform V. 3. 3 (Miller et al., 2010) with tree heuristic search using SPR, with 10 random starting trees, with model selection by the SMS algorithm under the Bayesian information criterion (BIC), with two simulation runs of the Markov chain Monte Carlo (MCMC), for 10 million generations, sampling every 100 generations, and with a 'burn-in' removal of 25%. Node supports were assessed in BI by Bayesian posterior probabilities (BPP). Effective Sample Sizes (ESS) of parameters were estimated using Tracer v1.7.1 (Rambaut et al., 2018) to assess sampling robustness. We considered values over 100 effectively independent samples sufficient.

Table 2. Accession numbers of sequences from GenBank used in our phylogenetic analyze using with 28S rRNA gene.

Class	Family	Species	Acession number	Reference
		Oligacanthorhynchus tortuosa 1	AY210466	Passamaneck and Halanych (2006)
		Oligacanthorhynchus tortuosa 2	KM659327	Lopez-Caballero et al. (2015)
		Macracanthorhynchus ingens	AY829088	Garcia-Varela and Nadler (2005)
Archiacanthocephala	Oligacanthorhynchidae	*Oncicola venezuelensis*	KU521567	Santos et al. (2017)
		Moniliformis moniliformis 1	AY829086	Garcia-Varela and Nadler (2005)
		Moniliformis moniliformis 2	MF398414	Mendenhall et al. (2018)
		Mediorhynchus sp.	AY829087	Garcia-Varela and Nadler (2005)
		Gigantorhynchus echinodiscus	**MK635344**	**present study**
Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus lucii*	AY829101	
	Plagiorhynchidae	*Plagiorhynchus cylindraceus*	AY829102	Garcia-Varela and Nadler (2005)
Eoacanthocephala	Neoechinorhynchidae	*Neoechinorhynchus saginata*	AY829091	
		Floridosentis mugilis	AY829111	

5.3 Results

5.3.1 Redescription

Family Gigantorhynchidae Hamann, 1892

Genus *Gigantorhynchus* Hamann, 1892

Gigantorhynchus echinodiscus (Diesing, 1851)

Body of median size and narrow. Sexual dimorphism in body size, with females larger than males. Proboscis cylindrical (Figures 1, 6 and 12) and similar in both sexes with a single crown of large hooks in the apex of the proboscis (Figures 6 and 8), formed by two rows of hooks in a total of 18 hooks with double roots (Figures 1, 8 and 12). The first row with six-robust hooks and the second row with 12 hooks in pairs, smaller than those in the first row (Figure 2 and 8). Measurement of the hooks with double root: from the tip of the hook to the hook root, total length of the hook blade; and total of the root: six hooks of the first row measured 0.16-0.23 (0.20); 0.12-0.18 (0.15); 0.11-0.16 (0.14). The 12 hooks of the second row measured 0.18-0.19 (0.18); 0.11-0.13 (0.12); 0.11-0.12 (0.11), respectively.The crown is separated from numerous small-rootless hooks by a slight space without hooks (Figure 6). The small-rootless hooks were arranged in longitudinal rows (Figure 1, 2, 6 and 7) and measured 0.05-0.08 (0.07). Two lateral papillae in the neck were observed with a slightly elevated border (Figure 1, 7 and 9). Behind the proboscis, it was observed a a smooth region. The lemnisci were long and filiform in both sexes.

Male (nine specimens): Body 14.80-45.29 (31.53) long and 0.53-0.99 (0.78) wide. Proboscis and neck 0.45-0.65 (0.55) long and 0.30-0.55 (0.45) wide having a crown with 18 hooks followed by numerous and small-rootless hooks arranged on longitudinal rows. After the proboscis a region without segmentation measuring 2.24-3.21 (2.72) long. The proboscis receptacle 0.48-0.64 (0.57) long and 0.21-0.32 (0.26) wide. The lemnisci 8.02-20.30 (14.87) (n=3), reaching the anterior testis. The testes were ellipsoids, narrow, and in tandem; the anterior testis 1.63-2.71 (2.25) long and 0.26-0.32 (0.29) wide; posterior testis 1.61-2.66 (2.13) long, and 0.26-0.39 (0.29) wide (Figure 3). Eight cement glands disposed in pairs, the group of cement glands measured 0.98-2.13 (1.61) long and 0.45-0.76 (0.60) wide (Figures 3 and 14) followed by an ejaculatory duct 0.82-1.42 (0.97) long. The posterior end after the

anterior testes have a smooth region, measured 5.45-8.53 (6.83) and had smooth surface with a copulatory bursa at the end (Figures 3 and 14).

Female (six specimens): Body 52.92-102.79 (75.45) long and 0.79-1.13 (0.85) wide. Proboscis and neck 0.49-0.71 (0.55) long and 0.46-0.53 (0.48) wide. Proboscis receptacle 0.63-0.74 (0.70) long and 0.23-0.31 (0.27) wide. The lemnisci were long and difficult to see due to be covered by eggs in most specimens and measured 13.23 mm long (n=1). Gonopore subterminal and vagina has sinuous lateral region in "guitar" format (Figures 4, 15, and 16). The distance from uterine bell to genital pore including the vagina, uterus, and uterine bell measured 0.69-0.97 (0.86) (n=5) (Figure 4). Eggs were ellipsoids with four membranes 0.059-0.069 (0.064) long and 0.04-0.03(0.036) wide (n=26; Figures 5 and 13).

Taxonomic summary

Host: *Myrmecophaga tridactyla* Linnaeus, 1758

Site: Small intestine.

Locality: Santa Bárbara Ecological Station – ECc Santa Bárbara (22°48'59″S, 49°14'12″W), São Paulo, Brazil.

Specimens deposited: CHIOC n°. 38580
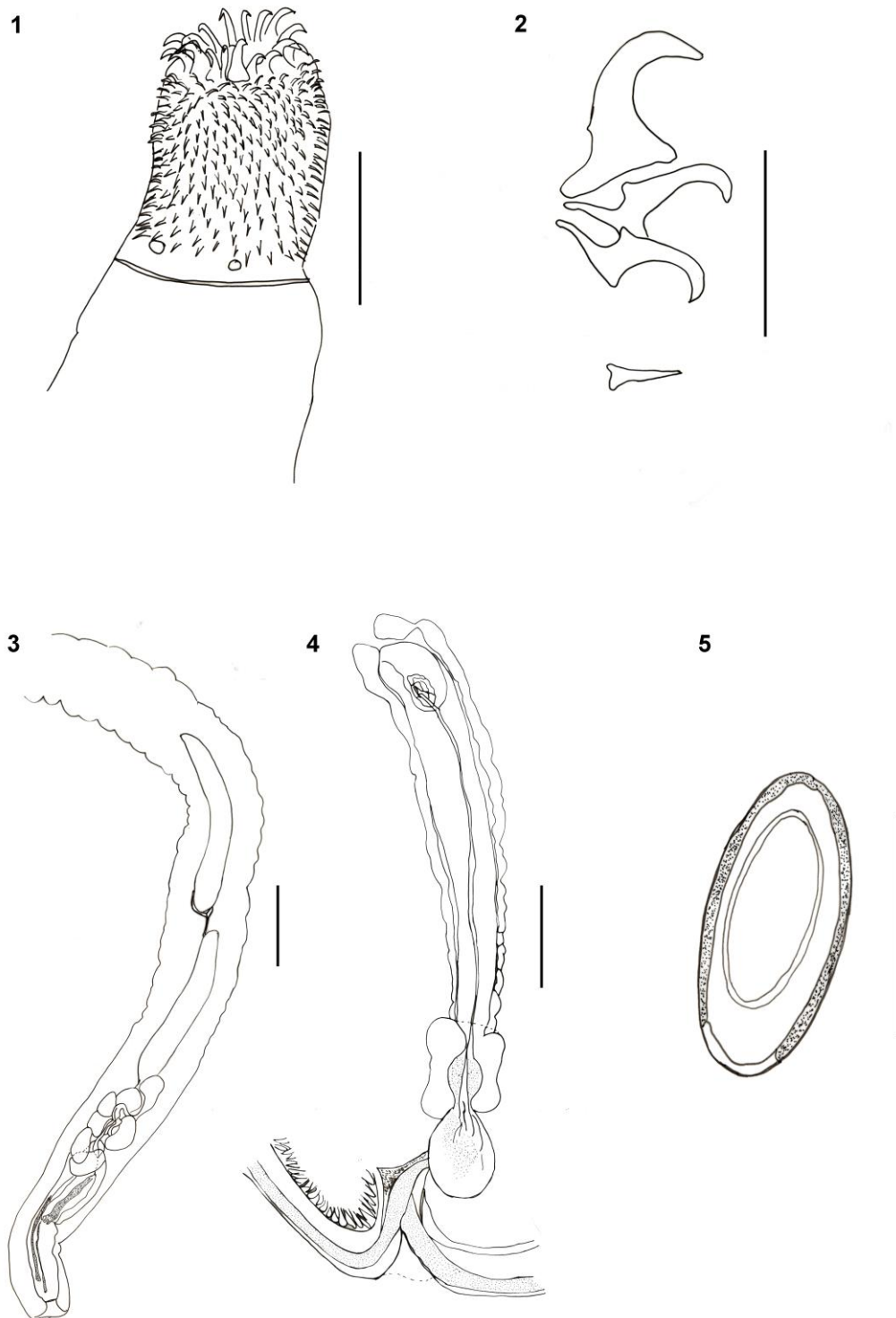
Figure 1-5. Line drawing *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 1. Praesoma with the proboscis presenting a crown with robust hooks followed by small hooks; 2. Three different robust hooks in the crown and a small one type in the proboscis; 3. Posterior region of adult male showing reproductive organs; 4. Posterior region of adult female showing the uterus, vagina and gonopore subterminal; 5. Egg (sacle bar=100µm).
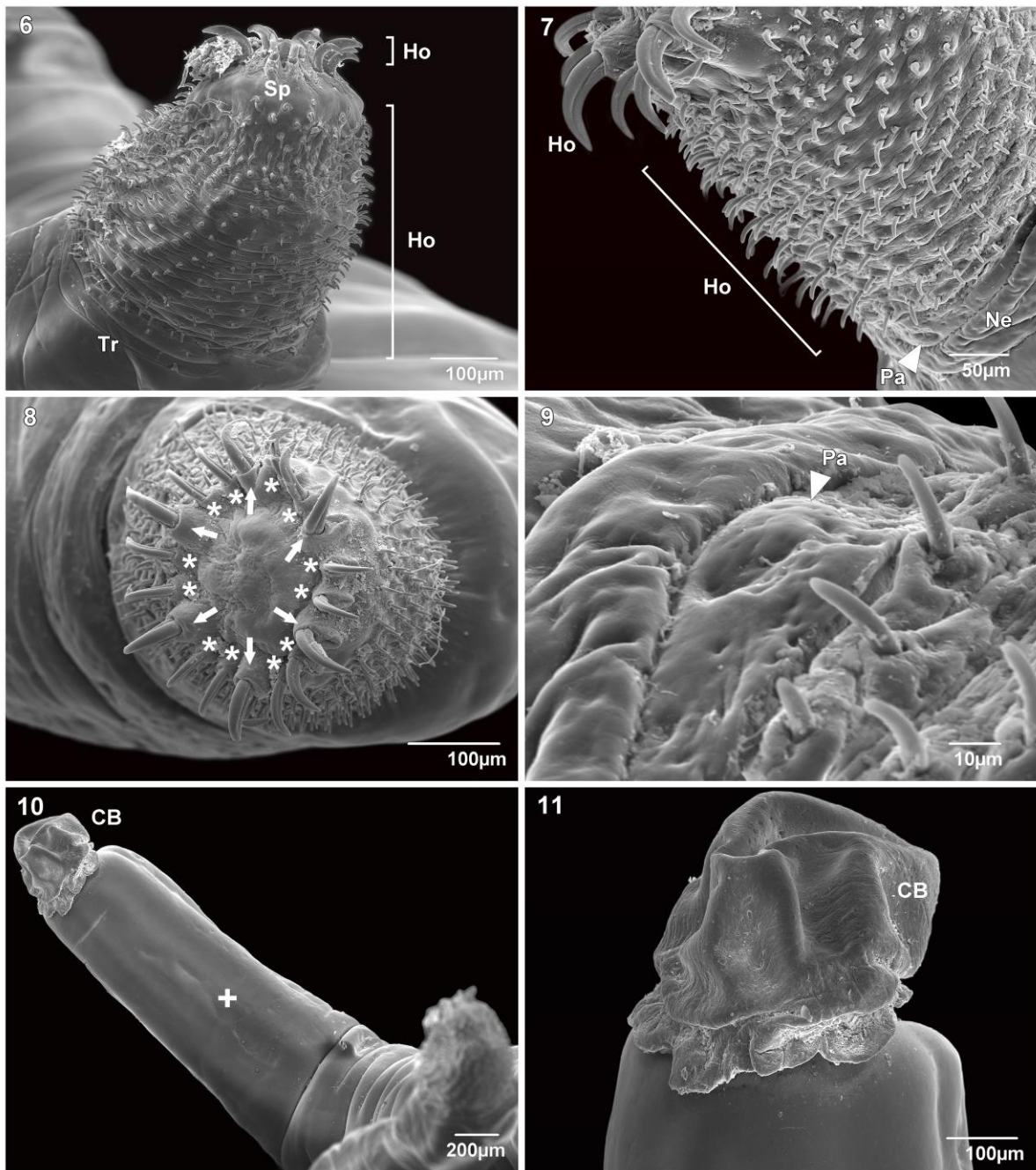
Figure 6-11. Scanning electron microscopy of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 6 and 7. Cylindrical proboscis armed with hooks (Ho) showing a space (Sp) between the two circles of large hooks and small rootless hooks, neck (Ne), trunk (Tr), lateral papillae (Pa); 8. Detail of the crown with two circles of large hooks; 9. Detail of the lateral papillae; 10 and 11. Posterior end of adult male showing the region without pseudo-segmentation (cross) and a copulatory bursa protruded body (Cb).
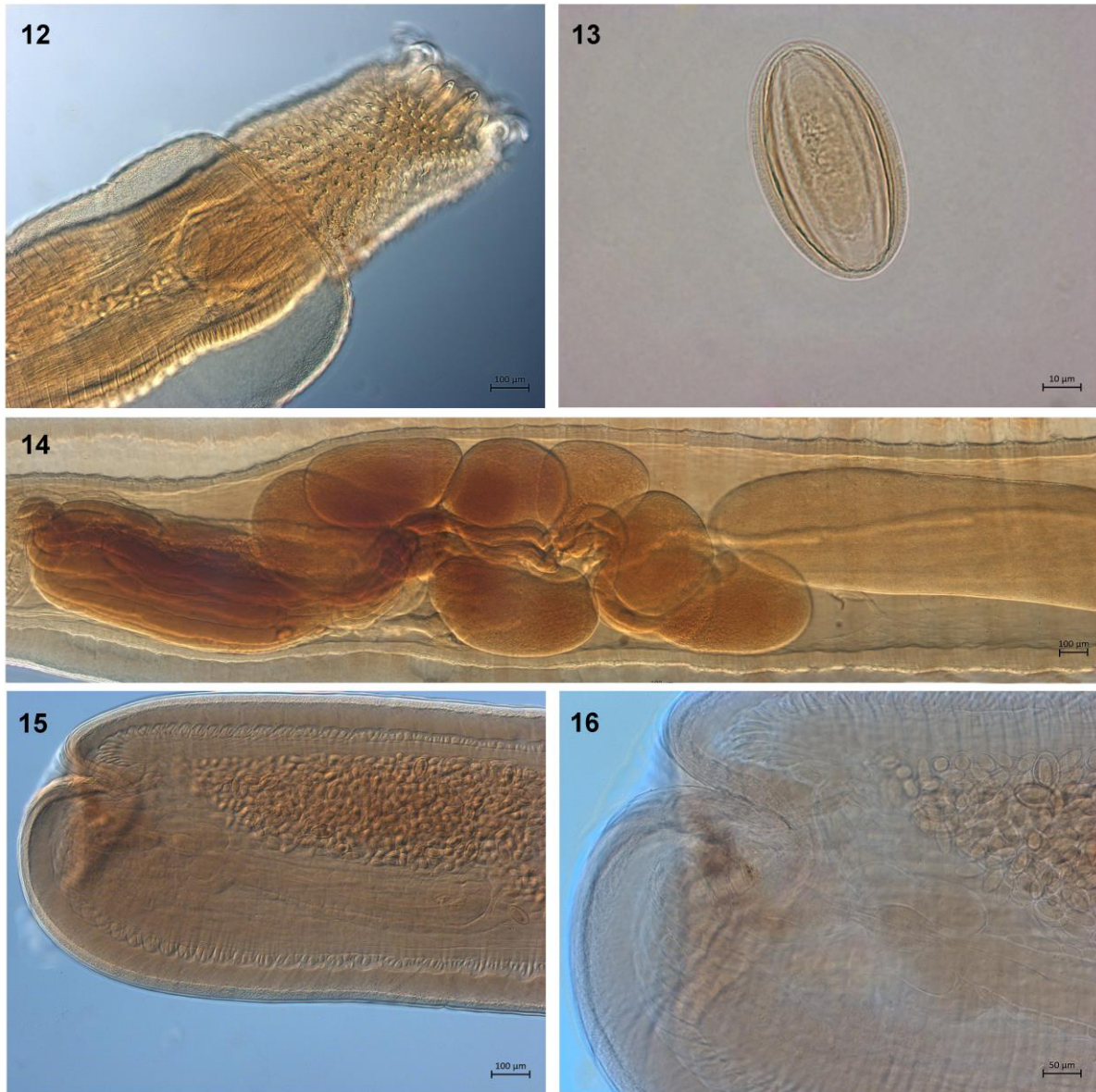
Figure 12-16. Light microscopy of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 12. Proboscis with a crown of large hooks in the apex and small hooks; 13. Egg; 14. Testis, cement glands in pair, ejaculatory duct; 15 and 16. Detail of the posterior end of adult female showing the uterus, vagina and gonopore subterminal.

5.3.2 Molecular analyses

Sequencing of partial 28S rRNA gene results in a consensus sequence of 771bp from one adult *Gigantorhynchus echinosdiscus* (Diesing, 1851)*.* The resulting matrix was comprised of 12 taxa and 534 characters, of which 68 characters were constant (proportion =0.1273), 194 were parsimony-uninformative and 272 were parsimony-informative variable characters. The PTP (P =0.0001) and the G1 (G1 =0.9227) tests indicated the presence phylogenetic signal and the test by Xia provided no evidence for substitution saturation in the 28S rRNA data matrix.

The MP analysis resulted in a 1053 steps length single most-parsimonious tree with 0.7179 consistency index (CI), 0.2821 homoplasy index (HI), and 0.3695 rescaled consistency index (RC). The ML best-fit model chosen by SMS on PhyML under AIC was the TN93+G, with 4 substitution rate categories, and gamma shape parameter 1.217, resulting in a tree with score lnL= -3556.2275. The best-fit model used to infer BI under BIC chosen by SMS on PhyML was HKY+G and the BI resulted in a mean estimated marginal likelihood -3571.9031 (median =3571.5520, standard deviation =39.3280). Estimated sample sizes (ESS) were robust for all parameters.

Our phylogenies inferred using MP, ML and BI resulted in similar topologies with variations in nodes and support values. The BI topology is shown in Figure 17. The class Archiacanthocephala was monoplyletic with strong support (MP-BP =0.97, aLRT =0.95, ML-BP =0.88, BPP =1.00). All analyses agreed that the sequence of *G. echinodiscus* formed a moderately to well-supported monophyletic group with *Mediorhynchus* sp. (MP-BP =0.68, aLRT =0.91, ML-BP =0.55, BPP =0.91). The family Gigantorhynchidae Hamann, 1892 (*Gigantorhynchus* Hamann, 1892 and *Mediorhynchus* Van Cleave, 1916) was sister to the family Moniliformidae Van Cleave, 1924 (MP-BP =0.67, aLRT =0.68, ML-BP =0.32, BPP =0.70) represented by sequences of *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 that formed a well-supported monophyletic group (MP-BP = 1.00, aLRT = 1.00, ML-BP = 1.00, BPP = 1.00). The group formed by Gigantorhynchidae and Moniliformidae was sister to a group formed by sequences of *Macracanthorhynchus ingens* (von Linstow, 1879) Meyer, 1932 and *Oncicola venezuelensis* Marteau, 1977 (MP-BP =0.54, aLRT =0.72, ML-BP =0.42, BPP =0.68), although with low support. In addition, the sequences of *Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972 formed a

well-supported monophyletic group (MP-BP =1.00, aLRT =0.99, ML-BP =1.00, BPP =1.00) sister to all the other archiacanthocephalans.
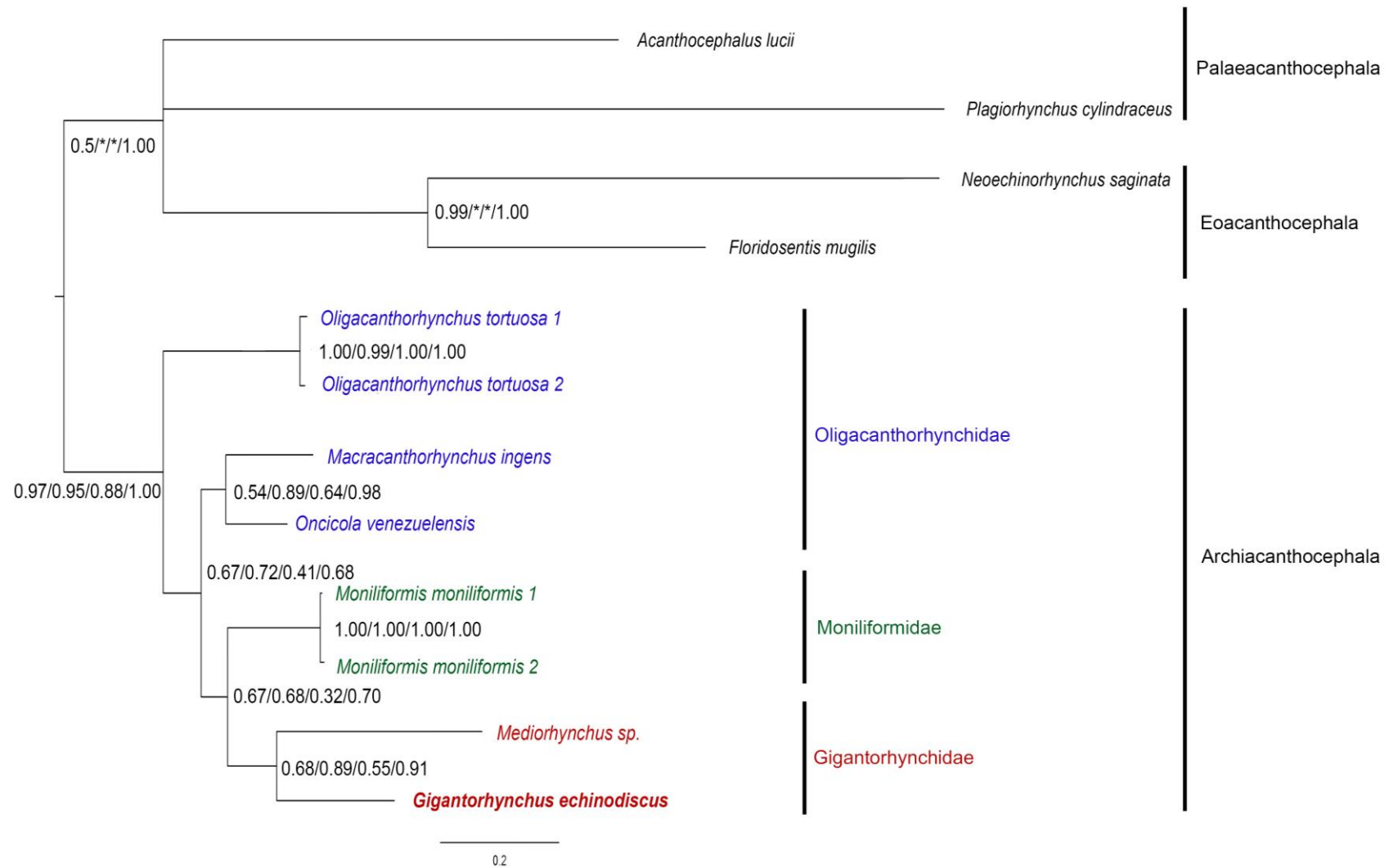
Figure 17. Bayesian Inference phylogenetic reconstruction tree of 28S rRNA gene sequences of *G. echinodiscus* (Diesing, 1851) in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups. Nodes values are MP-BP, aLRT, ML-BP, and BPP, respectively. (*) no support or node values were not recovered in the respective analysis.

5.3.3 Remarks

Species of the genus *Gigantorhynchus* are characterized by the presence of a cylindrical proboscis with a crown of robust hooks followed by numerous small hooks; long body with pseudo segmentation; lemnisci long and filiform; and ellipsoid testes (Travassos, 1917; Sothwell and Macfie, 1925, Yamaguti, 1963). The type hosts of the genus are marsupials and anteaters in South America (Travassos, 1917, Strong et al., 1926, Machado Filho, 1941, Sarmiento, 1954, Antonio, 1958, Díaz-Ungría, 1958). However, there is one report of infection of a baboon in Africa, *G. pesteri* (*nomen inquerendun*), which was considered to have uncertain taxonomic status due to a lack of some information such as the type host species, the registration number and deposit of the material in the collection, and the description was based in two immature females (Table 3). The taxonomy of this species needs to be revised.

The specimens we found parasitizing *M. tridactyla*, were identified as *G. echinosdiscus* due to the presence of a single crown with two rows of 6 and 12 hooks, totalling 18 hooks, ringed pseudo-segmented body, long testes, and eight cement glands in pairs. This species is distinguished from *G. lutzi*, *G. lopezneyrai*, *G. ortizi*, and *G. pesteri* by the number and size of hooks of the crown in the proboscis, type of pseudosegmentation, and size of the eggs (Table 3).

The number and the size of hooks on the proboscis of *G. echinosdiscus* in the present study was similar to that of *G. echinosdiscus* and *G. ungriai* described by Travassos (1917) and Antonio (1958), respectively. However, *G. echinosdicus* was distinguished from *G. ungriai* by the size of the proboscis, size of the hooks in the crown, and the type of segmentation, which has ringed complete segmentation with union in dorsal and ventral regions in *G. ungriai*, whereas *G. echinosdicus* lacks

ringed form with incomplete segmentation, as well as by the geographical distribution (Table 3).

Our specimens of *Gigantorhynchus echinodiscus* from *M. tridactyla* showed a similar morphology to the specimens described by Travassos (1917) and Diesing (1851), such as the number of the hooks in the crown, shape of the testes and cement glands, unsegmented region after the neck, lemnisci filiform, but showed little variation in morphometric analysis. Additionally, our study provides detailed information by SEM, such as the organization of the hooks in crown and the small hooks in the proboscis. We also found new information such as the space between the crown and the small hooks, the papillae at the end of the proboscis, as well as the unsegmented region with smooth surface in the posterior end of the male, and the shape of the copulatory bursa. These characteristics were not previously reported in the original description, especially in great detail by SEM for *G. echinodischus* and for other species of the *Gigantorhynchus* genus, offering more information of the type species and adding taxonomic information for future studies.

Table 3. Morphometric comparisons of *Gigantorhynchus* species (measurements in milimmiters).

Species	*Gigantorhynchus echinodiscus*		*Gigantorhynchus echinodiscus*		*Gigantorhynchus lutzi*		*Gigantorhynchus lopezneyrai*	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Trunk Length	50-75	150-220	18.0	-	35-60	130-200	16-58	-
Trunk Width	1-2.0	1.5-3.0	1.0	-	0.75-1.15	1-2.5	1-1.7	-
Anterior end without segmentation	4.0-5.0		3.0		-		no region without segmentation	
Proboscis+neck Length	1.0		1.0		1.695		1.131-1.5	
Proboscis+neck Width	0.5		0.735		0.66			
Number of hooks	18 (6+12)		18 (6+12)		12 (6+6)		12 (4+8)	
Hook to root x root	0.20 x 0.13 (1st row), 0.15 x 0.08 (2nd row)		0.18 (1st row) x 0.14 (2nd row)		0.285 x 0.165 (1st row), 0.225 x 0.135 (2nd row)		0.235 (1st row), 0.106 (2nd row)	
Small hooks length	0.04		0.04		0.048		-	
Receptacle	-		-		-		-	
Lemnisci	20-30		7.9-9.0		2.595		8	
Anterior testis Posterior testis	6-8.0 x 0.5-0.8		1.0 x 0.4		5.752-6.045 x 0.750-0.900		0.7 x 0.190	
Number of cement glands	8		8		8		8	
Dimension group of cement glands	4-5.0		-		-		-	
Organization of cement glands	in pairs		in pairs		in pairs		in pairs	
Ejaculatory duct	1.5-2.0		-		2.10-2.55		-	
Uterine bell	-		-		1.575 x 0.270		-	
Eggs	0.064 x 0.042		0.064-0.07 x 0.042-0.045		0.115 x 0.064		-	
Author	Travassos, 1917		Díaz-Ungría, 1958		Machado Filho, 1941		Díaz-Ungría, 1958	
Geographic distribuition	Rio de Janeiro, São Paulo, Brazil; Trinidad island; Panama; Venzuela		Atures, Venezuela		Pará, Brazil; Huanuco, Peru		Venezuela	
Vertebrate Host	*Tamandua tetradactyla, Cyclopes didactylus, Myrmecophaga tridactyla*		*Tamandua tetradactyla*		*Caluromys philander; Didelphis marsupialis*		*Tamandua tetradactyla*	
Reference	Travassos, 1917; Strong et al., 1926; Dunn, 1934; Camerón, 1939; Antonio, 1958		Díaz-Ungría, 1958		Machado Filho, 1941; Tantalean et al., 2005		Díaz-Ungría, 1958	

Table 3. Morphometric comparisons of *Gigantorhynchus* species (measurements in milimmiters).

Species	*Gigantorhynchus ortizi*		*Gigantorhynchus pesteri*		*Gigantorhynchus ungriai*		*Giganthorhynchus echinodiscus* (present study)	
Sex	Male	Female	Male	Female (immature)	Male	Female	Male	Female
Trunk Length	46-75	130-242	-	15-18	22-36	129-136	31.53	75.45
Trunk Width	1.4-1.92	1.5-2.0	-	0.8-0.9	0.78-1.58	1-1.6	0.78	0.85
Anterior end without segmentation					2-2.6		2.72	
Proboscis+neck Length	1.45-1.72		0.35		0.189-1.0		0.50	0.55
Proboscis+neck Width	0.435-0.555		0.1		0.237-0.7		0.30-0.52 (0.42)	0.48
Number of hooks	12 (6+6)		4		18 (6+12)		18 (6+12)	
Hook to root x root	0.160 x 0.10 (1st row), 0.140 x 0.09 (2nd row)		0.03		0.140-0.2 (1st row), 0.104-0.180 (2nd row)		0.20 (1st row) x 0.14 (1st row), 0.18 (2nd row) x 0.11 (2nd row)	
Small hooks length	0.05		0.015		0.02-0.06		0.07	
Receptacle	0.750-0.920		0.75 x 0.18-0.2		-		0.57 x 0.26	0.70 x 0.27
Lemnisci	5.48-6.80		3.6-4		1.75-3.27		14.87	
Anterior testis	1.98-3.0 x 0.56-0.96		-		2.0-5.6 x 0.395-0.474	-	2.25 x 0.29	
Posterior testis						-	2.13 x 0.29	
Number of cement glands	8		-		8	-	8	-
Dimension group of cement glands	-		-		0.869 x 0.1896	-	1.61 x 0.60	-
Organization of cement glands	in group		-		-	-	in pairs	-
Ejaculatory duct		-	-		2.6	-	0.97	-
uterine bell		-		2.2		-		0.86
eggs	0.079-0.085 x 0.049-0.054		-		0.04-0.06 x 0.04		0.064 x 0.036	
Author	Sarmiento, 1954		Tadros, 1966		Antonio, 1958		present study	
Geographic distribuition	Junin, Peru; Colombia		Rhodesia, South Africa		Venezuela		São Paulo, Brazil	
Vertebrate Host	*Metachirus nudicaudatus*		Baboon		*Tamandua tetradactyla*		*Myrmecophaga tridactyla*	
Reference	Sarmiento, 1954; Tantalean et al., 2005		Tadros, 1966		Antonio, 1958		present study	

5.4 Discussion

The genus *Gigantorhynchus* was erected by Hamman, 1892 as the single genus of the family Gigantorhynchidae with the type species *Gigantorhynchus echinodiscus* (syn. *Echinorhynchus echinosdiscus*) (Diesing, 1851). In 1917, Travassos revised the family Gigantorhynchidae and separated the family in two subfamilies: Gigantorhynchinae and Prosthenorchinae. The genus G*igantorhynchus* was included in the subfamily Gigantorhynchinae with four more genera: *Moniliformis* (Travassos, 1915), *Oligacanthorhynchus* (Travassos, 1915), *Empodius* (Travassos, 1916), and *Hamanniella* (Travassos, 1915), parasites of mammals and birds. Van Cleave (1923) reviewed Acanthocephala proposing a classification key to the genera considered valid, including the genus *Gigantorhynchus* that includes parasites of mammals from the Neotropical region. Later, Southwell and Macfie (1925) divided Acanthocephala in three sub-orders: Neoechinorhynchidea, Echinorhynchidea and Giganthorhynchidea the last having only the genus *Gigantorhynchus* with one species *Gigantorhynchus echinodiscus*. Meyer (1931), studying acanthocephalans from the Berliner Museum considered valid two more genera *Mediorhynchus* (Van Cleave, 1916) and *Empodius* (Travasso, 1915). However, Ward (1952) reviewed the acanthocephalans and moved *Heteracantorhynchus* Lundström, 1942 and excluded *Empodius* from the family Gigantorhynchidae. Thereafter, Van Cleave (1953) reporting acanthocephalans from North American mammals, considered the genus *Empodius* synonymous to the genus *Mediorhynchus* and established only two genera within the family Gigantorhynchidae: *Gigantorhynchius* and *Mediorhynchus*. Next, Yamaguti (1963) revised the classification of the family Gigantorhynchidae and reconsidered four genera within the family: *Gigantorhynchus*, *Empodius*, *Mediorhynchus, and Heteracanthorhynchus*, with *Gigantorhynchus* including five valid species. Golvan (1994) revised the nomenclature of the phylum Acanthocephala considering the geographical distribution as a taxonomic criterion and included more 24 species to the genus *Gigantorhynchus* as synonyms of different genera. Indeed, Amin (2013) recently updated the classification of family Gigantorhynchidae including two genera: *Gigantorhynchus* and *Mediorhynchus,* in agreement with Van Cleave (1953). In addition, he considered valid six species: *G. echinosdichus* (Diesing, 1851), *G. lutzi* Machado Filho (1941), *G. ortizi* Sarmiento

(1953), *G. ungriai* Antonio (1958),*G. lopezneyrai* Díaz-Ungría (1958) and *G. pesteri* Tadros (1966), parasites of mammals (anteaters, didelphid marsupials, and a baboon) from South America and South Africa.

Amato et al. (2014) reported, for the first time in Brazil, cystacanths of *G. echinosdiscus* infecting termites as intermediate hosts. Termites are nearly the entire portion of the giant anteater's diet (Rodrigues et al., 2008, Gaudin et al., 2018), suggesting that these arthropods are intermediate hosts of *G. echinosdiscus.*

Our molecular phylogenetic analyses, suggested that *G. echinosdiscus* (Diesing, 1851) Hamann, 1892 is closely related to *Mediorhynchus* sp. by forming a well-supported monophyletic group, and being consistent with morphological data that group these two genera within the family Gigantorhynchidae.

Furthermore, our phylogenetic analyses of the Archiacanthocephala genera agreed with previous studies recovering the family Gigantorhynchidae Hamann, 1892 as sister to Moniliformidae Van Cleave, 1924, although with moderate support values. Additionally, according to previous studies with other molecular markers, such as CO1 and 18S, without *Gigantorhynchus*, the genus *Mediorhynchus* is sister to genus *Moniliformis* (García-Varela and Nadler, 2005; Amin et al., 2013; García-Varela and Pérez-Ponce de León, 2015; Amin et al., 2016). Noteworthy, was the basal, non-monoplyletic Oligacanthorhynchidae, suggesting that relationships may not be well resolved within this group, and the characters differing this group may be plesiomorphic, requiring further thorough studies.

In conclusion, our 28S rRNA gene study provided the first DNA sequence and the first phylogenetic analyses for the genus *Gigantorhynchus*. Thus, extending knowledge about acanthocephalans from Brazilian mammals and emphasizing the importance of integrative taxonomic studies to clarify their taxonomy.

Acknowledgments

6 CHAPTER 4: A NEW ARCHIACANTHOCEPHALA, *MONILIFORMIS N. SP.* FROM THE WILD RODENT *NECROMYS LASIURUS* (CRICETIDAE: SIGMONDONTINAE) IN BRAZILIAN CERRADO.

Chapter 4

A new Archiacanthocephala, *Moniliformis n. sp.* from the wild rodent *Necromys lasiurus* Lund, 1840 (Cricetidae: Sigmondontinae) in South America.

Abstract

A new species of *Moniliformis* Travassos, 1915 (Moniliformidae: Acanthocephala) is described from the hairy-tailed Bolo Mouse *Necromys lasiurus* Lund, 1840 (Cricetidae: Sigmondontinae) in the Brazilian Cerrado biome, Uberlândia, Minas Gerais, Brazil. The specimens were described by light and scanning electron microscopy. In addition, molecular phylogenies were inferred from partial DNA gene sequence of the nuclear large subunit ribosomal RNA gene (28S rRNA) and partial mitochondrial cytochrome c oxidase subunit I gene (MT-CO1). The new species can be distinguished from other moniliformid species by the number of rows and the number of the hooks per rows; the size of the proboscis; the size of the eggs, the host, and geographical distribution. Molecular phylogenies showed that *Moniliformis n. sp.* form a well-supported monophyletic group with other sequences of *Moniliformis*, which agrees with the morphological studies, allocating the new species within the genus and the family Moniliformidae Van Cleave, 1924. The analyses of genetic distance demonstrated that *Moniliformis* n. sp. is a new taxon within the genus *Moniliformis*. In conclusion, the present work added morphological and molecular information of the new species and a new host for the genus.

Keywords: Acanthocephala, *Moniliformis*, hairy-tailed bolo mouse, Cerrado biome, phylogenetic relationship.

6.1 Introduction

The genus *Moniliformis*, proposed by Travassos (1915) has *Moniliformis moniliformis* (Bremser, 1811) as its type species. The genus comprises 17 species, which parasitize mammals and birds in different parts of the world (Amin et al., 2014, 2016, 2019, Martins et al., 2017); two of them parasitize Brazilian mammals: *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 and *Moniliformis travassoi* Meyer, 1932. *Moniliformis moniliformis* is cosmopolitan, infecting humans and non-humans wild and domestic mammal (Travassos, 1917; Amin, 1985; Berenji et al., 2007, Salehabadi et al., 2008). In Brazil, it has been reported infecting the rodents *Rattus rattus* Linnaeus, 1758 and *Rattus norvegicus* Berkenhout, 1769, and the bat *Phyllostomus hastatus* Pallas, 1767, in different regions (Travassos, 1917; Machado Filho, 1946; Gibson and McCarthy, 1987; Tietz Marques and Scroferneker, 2003; Araújo et al. 2014; Santos and Gibson, 2015; Simões et al., 2016). *Moniliformis travassoi* Meyer, 1932 has been reported infecting only the Norway rat *R. novergicus* in Brazil (Travassos, 1917; Machado Filho, 1946). In addition, studies of molecular phylogeny have been contributing to describe new species, revealing crypt species, reconstructing hypotheses of phylogenetic relationship and clarifying taxonomc problems e.g. family and genera levels. Molecular phylogenies including species of *Moniliformis* have been complementary the conventional taxonomy in studies of integrative taxonomy revealing new and crypt species (Amin et al., 2014; 2016; 2019).

Rodents are hosts of a great number of parasites, especially helminths (Jones et al., 2008; Meerburg et al., 2009; Hans et al., 2015). In Brazil, studies of taxonomy and ecology of helminths from rodents have been reported, especially nematodes (Vicente et al., 1997; Anderson et al., 2009, Costa et al., 2018, Simões et al., 2010, 2011, 2012, 2017; Cardoso et al., 2016, Tavares et al., 2017). However, mostly helminths studies from Brazilian rodents focus on ecology, and studies on acanthocephalans from these hosts are still scarce.

Necromys lasiurus (Lund, 1840) is a small terrestrial Sigmodontine (<80 g) (Rodentia: Cricetidae) which is broadly distributed in South America, ranging from the Atlantic coast, through central Brazil to south of the Amazon River, including north-eastern Argentina, extreme south-eastern Peru, Paraguay, and Bolivia (Redford and Eisenberg, 1999; Bonvicino et al., 2008). In Brazil, this sigmodontine rodent inhabits

the grasslands of Cerrado, Pantanal, Caatinga, and open areas in the Atlantic Forest biome (Bonvicino et al., 2008). This sigmodontinae is considered a generalist species, and its diet includes fruits, leaves, seeds, and invertebrates (Vieira et al., 2010; Redford and Eisenberg, 1999). Helminths described in *N. lasiurus*, nematodes are the most frequent and reported (Vicente et al., 1997; Anderson et al., 2009). However, there is no report about species of the genus *Moniliformis* in this host.

The present study reports a species of the genus *Moniliformis* in *N. lasiurus* from the Brazilian Cerrado biome and a new host for the genus. Description was based on morphology and molecular phylogenetic analyses.

6.2 Material and Methods

6.2.1 Field study and collection of acanthocephalan specimens

During an investigation of Hantaviruses cases, rodents were captured in the municipality of Uberlândia (18°55′07″S, 48°17′19″W) in the state of Minas Gerais, Southeastern Brazil, within the Cerrado biome. Specimens of *Necromys lasiurus* (Lund, 1840) were captured with Sherman® traps (3 × 3.75 × 12 inches) and Tomahawk® (16 × 5 × 5 inches) baited with a mixture of peanut butter, banana, oats and bacon. Trapping occurred between December 2011 and November 2012. Mammals were anesthetized; euthanatized, necropsied, and abdominal and thoracic cavities were examined for the presence of helminths. Permits for rodent capture and handling were issued by the Chico Mendes Institute for Biodiversity Conservation (*Instituto Chico Mendes de Conservação da Biodiversidade* - ICMBio) under authorization number 13373, followed the protocol and approved by the Ethics Committee on Animal Use of Oswaldo Cruz Institute (CEUA, *Instituto Oswaldo Cruz/FIOCRUZ-RJ*), according to licenses L-049/08 and 066/08.

6.2.2 Morphological analysis

Worms recovered were washed in saline solution to remove tissue debris and fixed 70% ethanol and taken to the Laboratory of Biology and Parasitology of Wild Mammals Reservoir (*Laboratório de Biologia e Parasitologia de Mamíferos Silvetres Reservatórios* - LABPMR). At the LABPRM, the acanthocephalan specimens used for morphological characterization were stained with acid carmine, destained in a solution of 2% hydrochloric acid (HCl) and 70% ethanol, dehydrated in a graded

ethanol series (70 to 100%), clarified in 90% phenol (modified from Amato, 1985), and analyzed using an Axion Scope A1 Light Microscope with Zeiss Scope Z1 light microscope (Zeiss, Göttingen, Germany). Drawings were made with the aid of camera lucida attached to a Nikon light microscope Model Eclipse E200MVR (Nikon Corporation, Tokyo, Japan). Measurements were in millimeters unless otherwise stated, range followed by mean within parentheses. Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (*Coleção Helmintológica do Instituto Oswaldo Cruz* - CHIOC), Rio de Janeiro, Brazil under the number CHIOC n° 38594 a-c.

For scanning electron microscopy (SEM) the specimens previously fixed in 70% ethanol were dehydrated in ascending ethanol series (80%, 90%, 100%), dried by the critical point method with CO_2, mounted with silver cellotape on aluminum stubs, and sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LV microscope (JEOL, Akishima, Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute (Plataforma de Microscopia Eletrônica Rudolf Barth/IOC- FIOCRUZ).

6.2.3 *Molecular phylogenetic analyses*

For genomic DNA recovery, acanthocephalans specimens preserved in 70 % ethanol were washed in ultrapure water for 24 hours at room temperature. Total genomic DNA was isolated from an individual worm using the QIAamp DNA mini Kit according to the manufacturer's protocol (Qiagen, Venlo, The Netherlands). DNA amplification by polymerase chain reaction (PCR) was conducted using two primer pairs: partial nuclear large subunit ribosomal RNA gene (28S rRNA) was amplified using the primers C1 5′-ACCCGCTGAATTTAAGCAT-3′ and D2 5′-TGGTCCGTGTTTCAAGAC-3′ (Hassouna et al., 1984 - modified from Chisholm et al., 2001); and partial mitochondrial cytochrome c oxidase subunit I gene (MT-CO1) using the primers F 5'-CTAATCATAARGRTATYGG-3' and R 5'-TAAACYTCAGGRTGACCAAARAAYCA-3' (Falla et al., 2015 - modified from Folmer et al.,1994). PCR amplifications were performed using Promega PCR Master Mix (Promega Corporation, Madison, Wisconsin, USA). Reactions were 25 μL, following the manufacturer's protocol. The thermal-cycling profiles were programmed on a thermocycler Eppendorf Mastercycler Epsystem (Eppendorf, Hamburg, Germany) and executed for 28S rRNA gene with an initial denaturation step of 95 °C/ 2 min;

followed by 40 cycles of 94 °C/ 60 s; 55 °C/ 60 s, and 72 °C/ 60 s; a final extension at 72 °C/ 5 min; and a rapid cool down to 4°C. PCR profiles, for MT-CO1 gene, consisted in an initial denaturation step at 95 °C/ 2 min; 35 cycles of 94 °C/ 1 min, 40°C/ 1 min, and 72 °C/ 1 min; followed by a final extension at 72 °C/ 5 min; and hold of 4°C. PCR products were analyzed after electrophoresis on 1.5% agarose gel using GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) by visualizing on UV transilluminator.

Successful amplifications were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Sequencing reactions using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) were performed using the same primers mentioned above in a Gene Amp (Applied Biosystems) thermocycler and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems). Both procedures and cycle-sequenced products precipitations were conducted at the subunit RPT01A – DNA sequencing platform of the Oswaldo Cruz Institute PDTIS/FIOCRUZ.

For each gene, chromatograms were initially assembled into contigs, and manually edited for ambiguities using the software package Geneious 9.1 (http://www.geneious.com; Kearse et al., 2012). The resulting consensus sequences were compared for similarities with sequences of the GenBank database using the BLAST (http://www.ncbi.nlm.nih.gov/BLAST.cgi) "Basic Local Alignment Search Tool" algorithm from the National Center for Biotechnology Information (NCBI).

For molecular phylogenetic analyses using 28S rRNA and MT-CO1 datasets, we added sequences of the class Archiacanthocephala representatives retrieved from GenBank. Three families, representing three different orders of archiacanthocephalans, were inclued in our datasets: Oligacanthorhynchidae Southwell et Macfie, 1925 (*Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972, *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos, 1917, *Macracanthorhynchus ingens* (von Linstow, 1879) Meyer, 1932, *Prosthenorchis sp., Prosthenorchis elegans* (Diesing, 1851) Travassos, 1915, *Oncicola sp, Oncicola venezuelensis* Marteau, 1977 and *Oncicola luehei* (Travassos, 1917) Schmidt, 1972); Moniliformidae Van Cleave, 1924 *(Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915*, Moniliformis kalahariensis* Meyer, 1931*, Moniliformis saudi* Amin et al., 2016*, Moniliformis cryptosaudi* Amin et al., 2019, and our new *Moniliformis* sequence); and Gigantorhynchidae Hamann, 1892 (*Mediorhynchus sp.* and

Mediorhynchus gallinarum (Bhalerao, 1937) Van Cleave, 1947). All of these genera infect mammals and *Mediorhynchus* may infect birds, as well. As outgroup we used representatives sequences of the classes Palaeacanthocephala and Eoacanthocephala (Table 1).

The 28S rRNA dataset was aligned using the MAFFT program under default parameters using Geneious, and manually edited by removing non-complementary regions. The dataset was posteriorly realigned using the Geneious alignment algorithm using as settings: global alignment with free end gaps, cost matrix of transition/tranversion (5.0/1.0) and penalty of 6.0 for both gap opening and extension; followed by manual edition, removing non-complementary regions. The MT-CO1 dataset was aligned using the MUSCLE program under default parameters using Geneious, and manually edited by removing non-complementary regions, followed by realignment of the sequences using the Translator X online software (Abascal et al., 2010). Final manual editing of poorly aligned regions was made with Mesquite 3.51 package (Maddison and Maddison, 2018).

For both matrices, substitution saturation was assessed using the DAMBE program Version 7.0.35 (Xia, X., 2017) via the Xia test (Xia et al., 2003; Xia and Lemey, 2009), performed on fully resolved sites only; and transitions and transversions versus JC69 genetic distances graphs (Jukes and Cantor, 1969). Substitution saturation tests and graphs were also performed separately for each codon position on the MT-CO1 matrix.

Phylogenetic reconstructions were carried out using Maximum Parsimony (MP), maximum-likelihood (ML), and Bayesian Inference (BI) methods, for each matrix (28S rRNA and MT-CO1). MP was carried out using PAUP 4.0a164 (Swofford, 2003) with heuristic search using starting trees via stepwise addition, with 100 random sequence addition replicates, holding 10 trees at each step, and tree bisection and reconnection (TBR) branch-swapping algorithm. Node support in MP was assessed by non-parametric bootstrap percentages (MP-BP) after 10,000 pseudoreplications. ML was carried out using PhyML 3.0 (Guidon et al., 2010) with heuristic search using subtree pruning and regrafting (SPR), with 10 random starting trees. Model selection was by the SMS algorithm (Smart Model Selection) (Lefort et al., 2017) under the Akaike information criterion (AIC). Node support in ML were assessed by approximate likelihood-ratio test (aLRT) for branches (Anisimova and Gascuel, 2006) and by non-parametric bootstrap percentages (ML-BP) after 1,000

pseudoreplications. BI was carried out using MrBayes version 3.2.6 (Ronquist et al., 2012) on the CIPRES Science Gateway platform V. 3. 3 (Miller et al., 2010) with two simulation runs of the Markov chain Monte Carlo (MCMC), for 10 million generations, sampling every 100 generations, and with a 'burn-in' removal of 25%. Nucleotide substitution model was GTR+I+G on 28S rRNA matrix. To account for differences between codon positions independent GTR+I+G models were adopted for each codon position with unlinking of base frequencies and parameters. Node supports were assessed in BI by Bayesian posterior probabilities (BPP). Effective Sample Sizes (ESS) of parameters were estimated using Tracer v1.7.1 (Rambaut et al., 2018) to assess sampling robustness. We considered values over 100 effectively independent samples sufficient.

Additionally, to assess the level of variation in MT-CO1 among sequences of the matrix of different taxa, it was determined using the maximum likelihood genetic distance method in PAUP* 4.0a164 programm (Swofford, 2003).

Table 1. Classes, families, species, acession numbers and references of sequences from GenBank used in our phylogenetic analyses with 28S rRNA and Mt-CO1.

Classe	Family	Species	28S	MT-CO1	References
Archiacanthocephala	Oligacanthorhynchidae	*Oligacanthorhynchus tortuosa* 1	AY210466	KM659328	Passamaneck and Halanych, 2006; Lopez-Caballero et al., 2015
		Oligacanthorhynchus tortuosa 2	KM659327	AF416999	Lopez-Caballero et al., 2015; Garcia-Varela et al., 2016 (unpublished)
		Oligacanthorhynchus tortuosa 3	-	KT881245	Richardson et al., 2016 (unpublished)
		Macracanthorhynchus ingens	AY829088	AF416997	Garcia-Varela and Nadler, 2005; Garcia-Varela et al., 2016 (unpublished)
		Macracanthorhynchus hirudinaceus	-	LC350021	Kamimura et al., 2018
		Oncicola venezuelensis	KU521567	-	Santos et al. (2017)
		Oncicola sp.	-	AF417000	Garcia-Varela et al., 2017 (unpublished)
		Oncicola luehei	-	JN710452	Gazi et al., 2012
		Prosthenorchis sp.	-	KP997253	Sokolov et al., 2016 (unpublished)
		Prosthenorchis elegans 1	-	KT818500	Falla et al., 2015
		Prosthenorchis elegans 2	-	KT818501	Falla et al., 2015
	Moniliformidae	*Moniliformis moniliformis* 1	AY829086	AF416998	Garcia-Varela and Nadler, 2005; Garcia-Varela et al., 2016 (unpublished)
		Moniliformis moniliformis 2	MF398414	-	Mendenhall et al. (2018)
		Moniliformis n.sp.	-	-	**present study**
		Moniliformis kalahariensis	-	MH401040	Amin et al., 2019
		Moniliformis saudi	-	KU206783	Amin et al., 2016
		Moniliformis cryptosaudi	-	MH401041	Amin et al., 2019
	Gigantorhynchydae	*Mediorhynchus sp.* 1	AY829087	AF416996	Garcia-Varela and Nadler, 2005; Garcia-Varela et al., 2016 (unpublished)
		Mediorhynchus sp. 2	-	KC261351	Amin et al., 2013
		Mediorhynchus gallinarum	-	KC261352	Amin et al., 2013
Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus lucii*	AY829101	-	Garcia-Varela and Nadler, 2005
	Plagiorhynchidae	*Plagiorhynchus cylindraceus*	AY829102	-	Garcia-Varela and Nadler, 2005
	Plagiorhynchidae	*Plagiorhynchus transversus*	-	KT447549	Gazi et al., 2016
	Centrorhynchidae	*Centrorhynchus aluconis*	-	NC029765	Gazi et al., 2016
Eoacanthocephala	Neoechinorhynchidae	*Floridosentis mugilis*	AY829111	-	Garcia-Varela and Nadler, 2005
	Tenuisentidae	*Paratenuisentis ambiguus*	-	FR856885	Weber et al., 2013
	Quadrigyridae	*Pallisentis celatus*	-	JQ943583	Pan and Nie, 2013
	Polyacanthorhynchidae	*Polyacanthorhynchus caballeroi*	-	KT592358	Gazi et al., 2016

6.3 Results

6.3.1 Description

Family Moniliformidae Van Cleave, 1924

Genus *Moniliformis* Travassos, 1915

Moniliformis n. sp

Medium-sized worms with long body, small proboscis with numerous small hooks (Figs. 1, 7 and 13). Proboscis cylindrical, retractile and armed with 12 rows of 9-10 rooted hooks (Figs. 1 and 13). On the top of the proboscis no sensory pore were observed (Figs. 9 and 10). Hooks are similar in both sexes and recurved with a single roots (Figs. 2, 10 and 11). Proboscis receptacle were double walled and have muscles fibers arranged spirally (Fig 1). Neck absent. The lemisnci were long, flat, usually in middle of the body (Fig. 3).

Male (based on four mature adult specimens): Body 16.11-43.45 (30.54) long by 0.92-1.21 (1.04) width. Proboscis 0.30-0.45 (0.37) long and 0.14-0.24 (0.19) wide having 12 rows of nine to ten hooks rooted each. The proboscis receptacle 0.59-0.69 (0.64) by 0.21-0.26 (0.23). The leminisci 7.95 (n=1) long almost in the middle of the body and nucleated. Reproductive system at posterior end of trunk. The testes were ellipsoids, and in tandem; the anterior testis 2.29-2.45 (2.35) by 0.53-0.61 (0.58); posterior testis 1.55-2.24 (2.01) by 0.53-0.66 (0.58) (Fig. 4). Eight cement glands in pairs and compacted group after the posterior testis, the group measuring 0.91-1.26 (0.50) by 0.37-0.63 (0.50) (Fig. 4) followed by an ejaculatory duct 1.00-1.32 (1.18). Bursa at the end of the body were retracted in all specimens.

Female (based on five mature specimens): Body 26.08-40.84 (30.68) long by 0.92-1.66 wide. Proboscis with 12 rows of nine to ten hooks each, measure 0.40-0.43 (0.41) by 0.11-0.16 (0.13). The proboscis receptacle 0.66-0.71 (0.69) by 0.25-0.27 (0.26). The leminisci 6.26 long (n=1) mostly covered by eggs. The distance from uterine bell to genital pore including the vagina, uterus, and uterine bell measured 1.33-1.39 (1.36) (n=2) (Fig. 5). Eggs were ellipsoids with three membranes and measured 0.084-0.103 (0.094) long and 0.043-0.070 (0.052) wide (n=28; Figs. 6 and 14). The gonopore was terminal (Fig.12).

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Taxonomic summary

Type host: *Necromys lasiurus* (Lund, 1840)

Type locality: Uberlândia (18°55′07″S, 48°17′19″W), Minas Gerais, Brazil

Site of infection: Small intestine

Type material: CHIOC 38594 a-c (hollotype – a; allotype – b; paratypes – c)

Prevalence: 6.86%

Intensity: 10.29
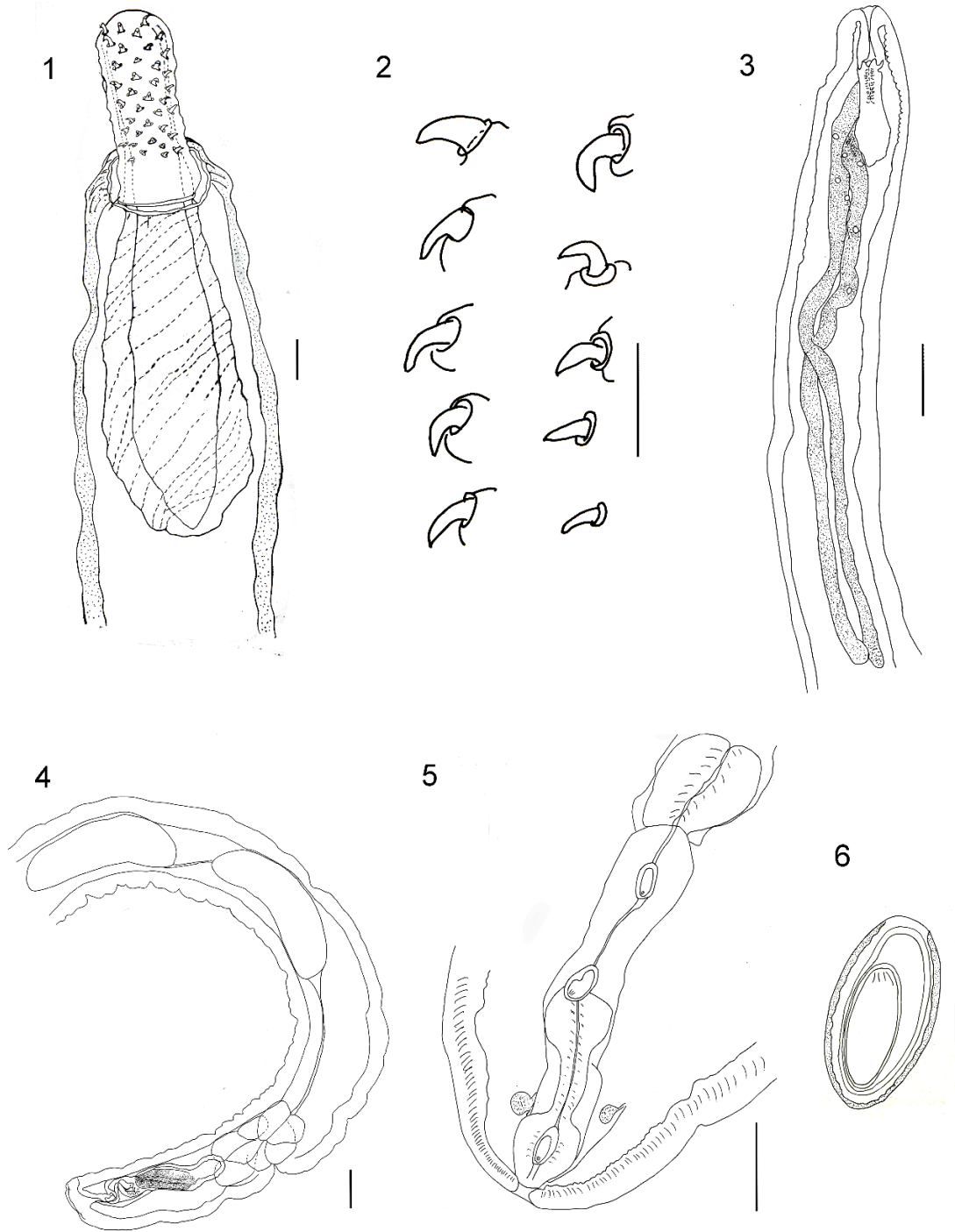
Figure 1-6. Line drawing of *Moniliformis n. sp.* from *Necromys lasiurus*. 1. Anterior region presents a cylindrical proboscis armed with small hooks, followed by a receptacle proboscis; 2. Small hooks from proboscis; 3. Leminisci flat, usually in middle of the body; 4. Male body with anterior and posterior testis, with 8 cement glands; 5. Posterior end of female body; 6. Ellipsoid eggs with three membranes (scale bar 100μm).
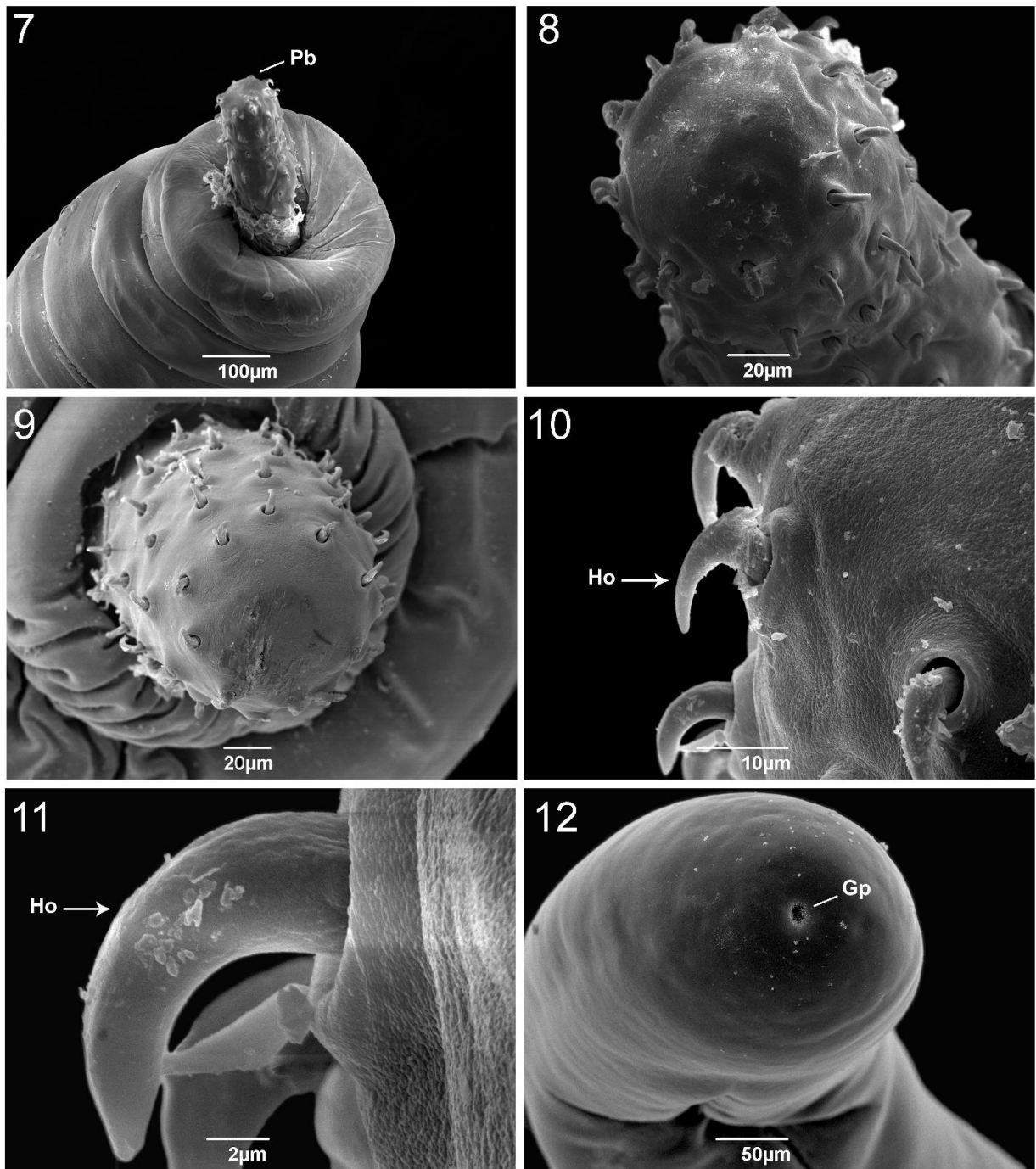
Figure 7-12. External morphology of *Moniliformis* n. sp. via scanning electron microscopy (SEM). 7. Proboscis armed with small hooks; 8 and 9. Apical view of the proboscis without sensory pore in apex of the proboscis; 10 and 11. Lateral view of anterior hooks of the proboscis; 12. Posterior end of adult female showing a terminal gonopore. Pb-proboscis, Ho-hook, Gp-gonopore.
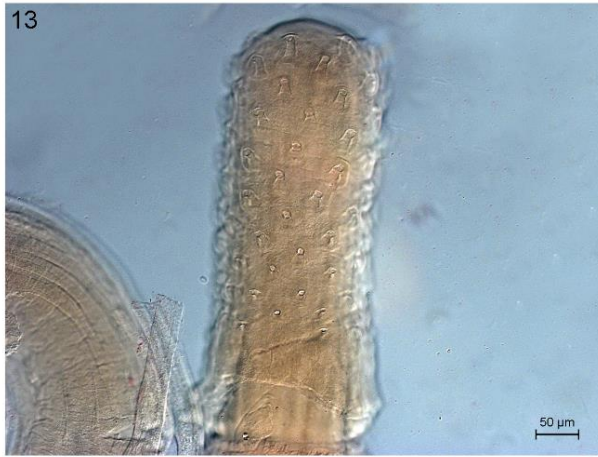
Figure 13-14. Light microscopy of adult *Moniliformis n. sp.* from *Necromys lasiurus*. 13. Cylindrical proboscis with small hooks; 14. Egg.

6.3.2 Molecular analysis

6.3.2.1 Phylogenetic analyses of 28S rRNA dataset

Our sequences resulted in a partial 28S rRNA gene consensus sequence of 760pb from one adult *Moniliformis n. sp.* The 28S rRNA resulting matrix was comprised of 11 taxa and 520 characters of which 189 characters were constant (proportion = 0.3635), 141 were parsimony-uninformative and 190 were parsimony-informative variable characters. The test by Xia provided no evidence for substitution saturation in the 28S rRNA data matrix (Table 2), likewise observed in the graph below (Fig. 15).
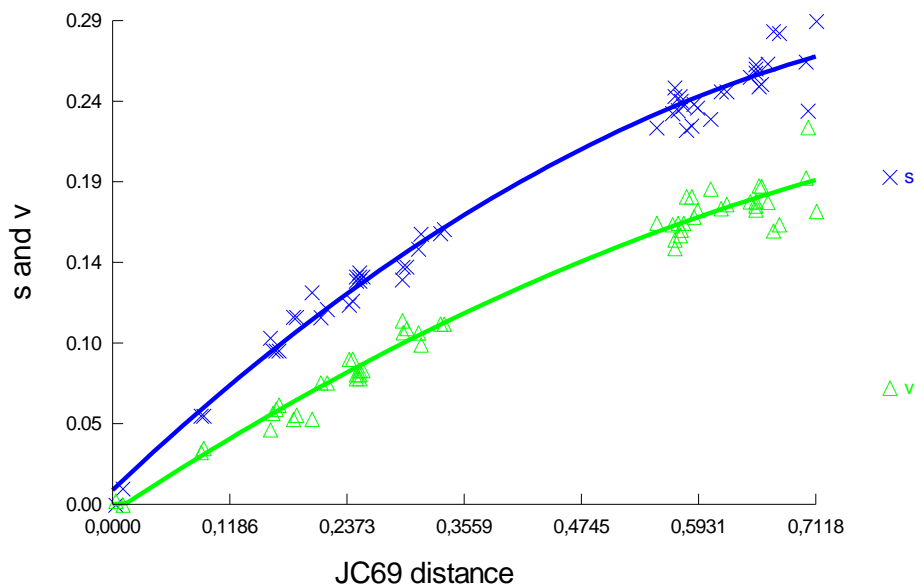
Figure 15. Transitions (s) and transversions (v) versus JC69 genetic distances graph of the 28S rRNA gene in acanthocephalan matrix.

Table 2. Index of substitution saturation (ISS) and critical ISS (ISSc), their respective p-values (P) under two tailed tests for symmetrical (Sym) and asymmetrical (Asym) trees in the 28S rRNA, MT-CO1, and the codon-wise partitioned MT-CO1 matrices.

	ISS	ISSc (Sym)	P	ISSc (Asym)	P
28 S rRNA	0.3769	0.7069	0.0000	0.5532	0.0000
MT-CO1	0.4428	0.7370	0.0000	0.4773	0.1767
MT-CO1 1st position	0.3876	0.6029	0.0000	0.3748	0.7494
MT-CO1 2nd position	0.2091	0.6029	0.0000	0.3748	0.0000
MT-CO1 3rd position	0.7696	0.6029	0.0000	0.3748	0.0000

The MP analysis resulted in a single 658 steps length most-parsimonious tree with 0.7219 consistency index (CI), 0.2781 homoplasy index (HI), and 0.4110 rescaled consistency index (RC). The ML best-fit model chosen by SMS on PhyML under AIC was the TN93+G, with four substitution rate categories, and gamma shape parameter 1.016, resulting in a tree with score lnL= -3049.6743. The substitution model used to infer BI was GTR+I+G, and the BI resulted in a mean estimated marginal likelihood – 2964.8606 (mean= -2964.521, standard deviation= 40.623). Estimated sample sizes (ESS) were robust for all parameters (ESS mean= 38482.4).

The 28S rRNA MP, ML, and BI tree topologies were similar with little variation in nodes and support values (Fig. 16 A-C; MP not shown). The class Archiacanthocephala sequences formed a well-supported monophyletic group (MP-BP= 1.00, aLRT= 0.79, ML-BP= 1.00, BPP= 1.00). All analyses also agreed that 28SrRNA sequences formed well-supported monophyletic groups with the two sequences of *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 (MP-BP= 1.00, aLRT= 0.61, ML-BP= 0.99, BPP= 1.00), and the sequence of *Moniliformis* n. sp, which the species of the present study is a sister to the other sequences of *Moniliformis moniliformis* with high support values (MP-BP= 1.00, aLRT= 0.65, ML-BP= 0.95, BPP= 1.00), these sequences representing the family Moniliformidae. The family Moniliformidae was sister to the family Oligacanthorhynchidae (MP-BP= 0.59, aLRT= 0.72, ML-BP= 0.45, BPP= 0.82) represented by sequences of *Macracanthorhynchus ingens* (von Linstow, 1879) Meyer, 1932 and *Oncicola venezuelensis* Marteau, 1977 (aLRT= 0.70, ML-BP= 0.43, BPP= 0.57), that formed a well-supported monophyletic group, although with low support. The group formed by

Moniliformidae and Oligacanthorhynchidae was sister Gigantorhynchiadae, represented by the sequence of *Mediorhynchus* sp. Van Cleave, 1916 also with low support (aLRT= 0.76, ML-BP= 0.37, BPP= 0.61).

In addition, the sequences of *Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972 formed a well-supported monophyletic group (MP-BP = 1.00, aLRT = 0.64, ML-BP = *, BPP = 1.00) sister to all the other archiacanthocephalans.

Figure 16 A. ML aLRT phylogenetic reconstruction tree of 28S rRNA gene sequences of *Moniliformis* n. sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups.

Figure 16 B. ML-BP phylogenetic reconstruction tree of 28S rRNA gene sequences of *Moniliformis* n. sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups. (* no support or node support values not recovered in the respective analysis).

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Figure 16 C. BPP phylogenetic reconstruction tree of 28S rRNA gene sequences of *Moniliformis* n.
sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups.

6.3.2.2 *Phylogenetic analyses of MT-CO1 dataset*

Our sequences resulted in a partial MT-CO1 gene consensus sequence of 706pb from one adult *Moniliformis* n. sp*.* Alignment of sequences resulted in a matrix comprising 23 taxa and 624 characters, of which 184 were constant (proportion = 0.2949), 60 were parsimony-uninformative, and 380 were parsimony-informative variable characters. The test by Xia and Lemey (2009) for substitution saturation provided evidence or saturation only at the third codon positions, whereas overall there was little saturation in the matrix (Table 2). Likewise it was observed in the graphs below (Figs.17-19).
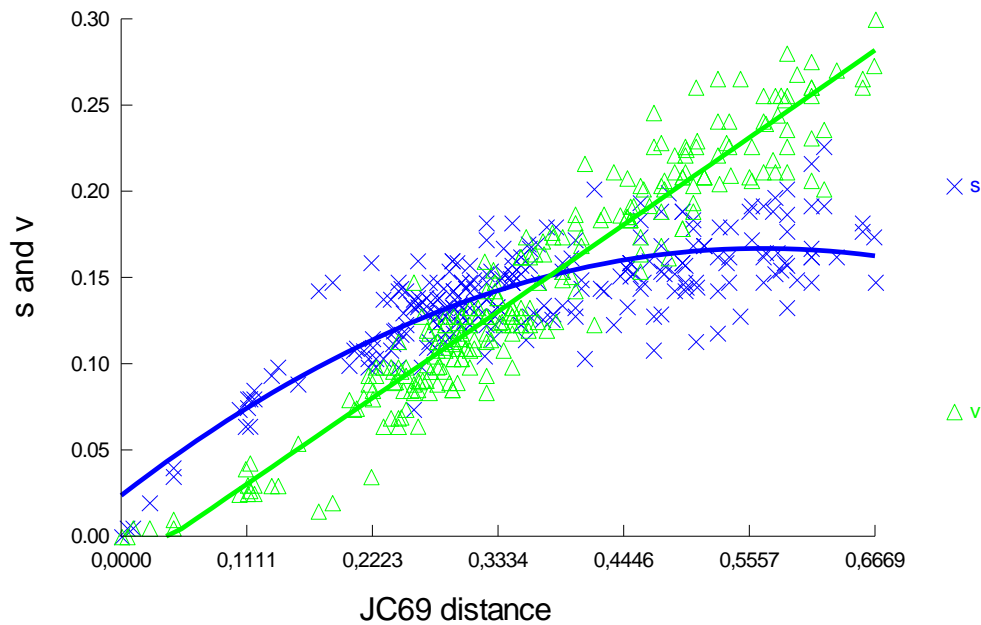
Figure 17. Transitions (s) and transversions (v) versus JC69 genetic distances graph of the first codon position of MT-CO1 gene in acanthocephalan matrix.
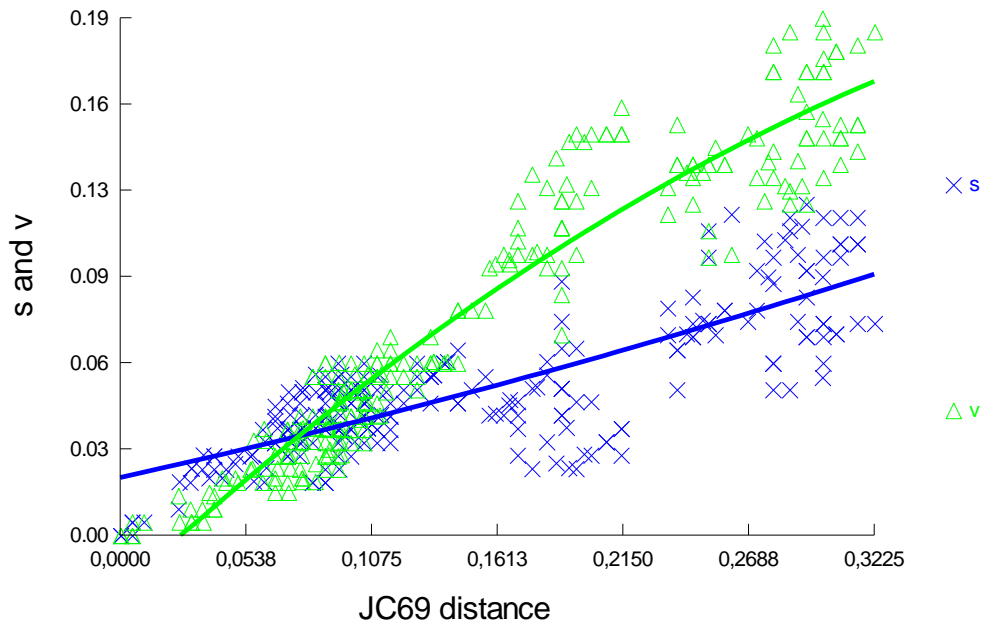
Figure 18. Transitions (s) and transversions (v) versus JC69 genetic distances graph of the second codon position of MT-CO1 gene in acanthocephalan matrix.
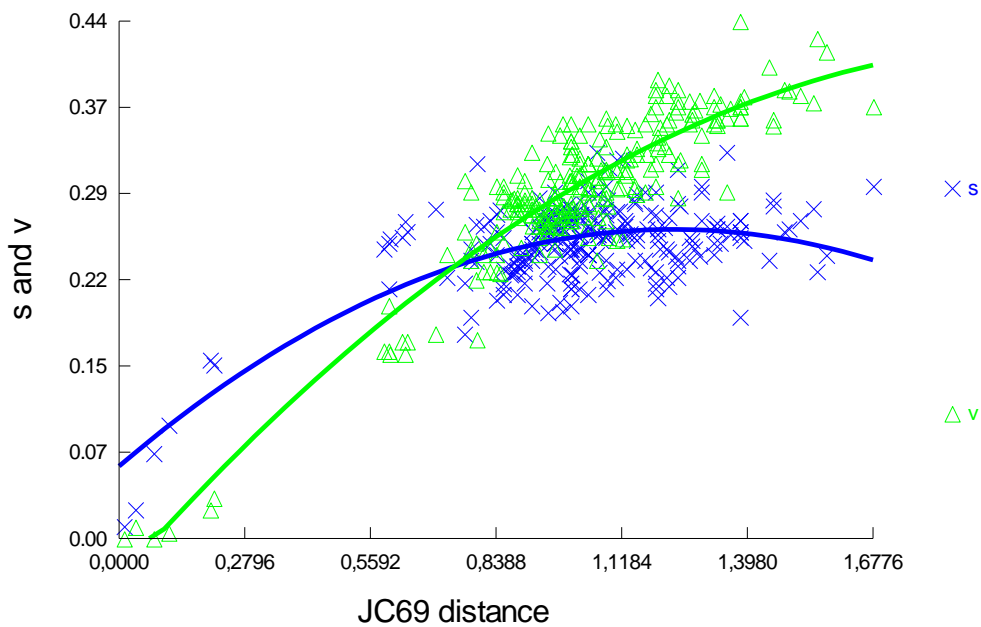
Figure 19. Transitions (s) and transversions (v) versus JC69 genetic distances graph of the third codon position of MT-CO1 gene in acanthocephalan matrix**.**

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The MP analysis resulted in a single 2114 steps length most-parsimonious tree with 0.4115 consistency index (CI), 0.5885 homoplasy index (HI), and 0.1942 rescaled consistency index (RC). The ML best-fit model chosen by SMS on PhyML under AIC was the GTR+G+I, with four substitution rate categories, and gamma shape parameter 0.641, resulting in a tree with score lnL= -8378.5516. For the BI analysis, the substitution model used was GTR+I+G the mean estimated marginal likelihood was -7954.7109, the median was -7954.3670, and standard deviation was 65.085. ESSs for all parameters were above 1000 effectively independent samples and for most parameters, indicating the robustness of our sampling (ESS mean= 26277).

MP, ML, and BI phylogenies resulted in similar topologies with little variation in nodes and support values, as shown in Figure 20 A-C (MP tree not shown). In all topologies, the MT-CO1 sequences of the genus *Moniliformis* formed a monophyletic group, having four well to moderate-supported group, although only moderately supported representing the family Moniliformidae. The sequence of species *Moniliformis n. sp.* was sister the sequences of *Moniliformis saudi* Amin et al., 2016, and *Moniliformis cryptosaudi* Amin et al., 2019, although poorly supported (MP-BP< 0.50, aLRT= 0.64, ML-BP= 0.54, BPP= 0.61); these last two formed a highly-supported group (MP-BP= 1.00, aLRT= 1.00, ML-BP= 1.00, BPP= 1.00). *Moniliformis kalahariensis* Meyer, 1931 and *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 sequences showed detached branches, which *M. kalahariensis* suggest as a sister with the group formed by *M. saudi*, *M. cryptosaudi* and *Moniliformis* n. sp (MP-BP = 0.60, aLRT = 0.92, ML-BP = 0.80, BPP = 0.66) with moderate nodal support. *M. moniliformis* sequences branches off separately from the other sequences in all phylogenetic analysis (MP-BP = *, aLRT = 0.82, ML-BP = 0.46, BPP = 0.84). The family Moniliformidae was sister to the family Oligacanthorhynchidae (MP-BP = *, aLRT = 0.53, ML-BP = 0.24, BPP = 0.68), although poorly supported, represented by sequences of three genera *Oncicola* Travassos, 1916, *Prosthenorchis* Travassos, 1915, *Macracanthorhynchus* Travassos, 1917. The sequences of the genus *Oncicola* represented by the sequences *Oncicola* sp. and *Oncicola luehei* (Travassos, 1917) Schmidt, 1972 formed a well-supported monophyletic group (MP-BP= 1.00, aLRT= 0.98, ML-BP= 1.00, BPP= 1.00) and sister of the genus *Prosthenorchis* (MP-BP= 0.99, aLRT= 0.99, ML-BP= 0.99, BPP= 1.00). The genus *Prosthenorchis* also formed a well-supported monophyletic group represented by the

sequences of *Prosthenorchis* sp. and two sequences of *P. elegans* (Diesing, 1851) Travassos, 1915 (MP-BP= 1.00, aLRT= 0.86, ML-BP= 0.91, BPP= 0.99), which the sequences of *P. elegans* formed a clade (MP-BP= 1.00, aLRT= 0.88, ML-BP= 0.91, BPP= 0.97) that was sister of the sequence *Prosthenorchis* sp. The group formed by sequences of the genera *Oncicola* and *Prosthenorchis* was sister to the sequence of the genus *Macracanthorhynchus* (aLRT = 0.83, ML-BP = 0.48, BPP = 0.99) represented by sequences of *M. hirudinaceus* (Pallas, 1781) Travassos, 1917 and *M. ingens* (von Linstow, 1879) Meyer, 1932, that formed a clade with high supported value (aLRT= 0.90, ML-BP= 0.67, BPP= 0.99), however in MP tree showed as polyphyletic sequences. The sequences of *Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972, which also representing the family Oligacanthorhynchidae formed a well-supported monophyletic group (MP-BP= 1.00, aLRT= 0.99, ML-BP= 1.00, BPP= 0.81), and sister to the family Moniliformidae and the other sequences of the family Oligacanthorhynchidae. In addition, the sequences of the genus *Mediorhynchus* Van Cleave, 1916 represented by the two sequences of *Mediorhynchus* sp. and *M. gallinarum* (Bhalerao, 1937) Van Cleave, 1947 formed a well-supported monophyletic group (MP-BP = 0.85, aLRT = 0.86, ML-BP = 0.45, BPP = 1.00), and sister to all the other archiacanthocephalans.

The ML- distances pairwise for representative's sequences of three classes of acanthocephalans Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala are provided in Table 3. Our matrix had ML- distances pairwise ranging from 0.844 between *Monilformis moniliformis* (Archiacanthocephala) and *Pallisentis celatus* (Eoacanthocephala) to 0.003 distances within *Moniliformis cryptosaudi* and *Moniliformis saudi* (mean= 0.485).

MT-CO1 sequence ML- distances of Archiacanthocephala (ingroup) and Palaeacanthocephala + Eoacanthocephala (outgroup) ranged from 0.845 between *Monilformis moniliformis* and *Pallisentis celatus* to 0.491 between *Plagiorhynchus transversus* and *Monilformis kalahariensis* (mean= 0.656). Within the class Archiacanthocephala the genetic ML- distances ranged from 0.542 between *Oligacanthorhynchus tortuosa* and *Mediorhynchus* sp. 1 to 0.003 between *Moniliformis cryptosaudi* and *Moniliformis saudi* (mean= 0.377).
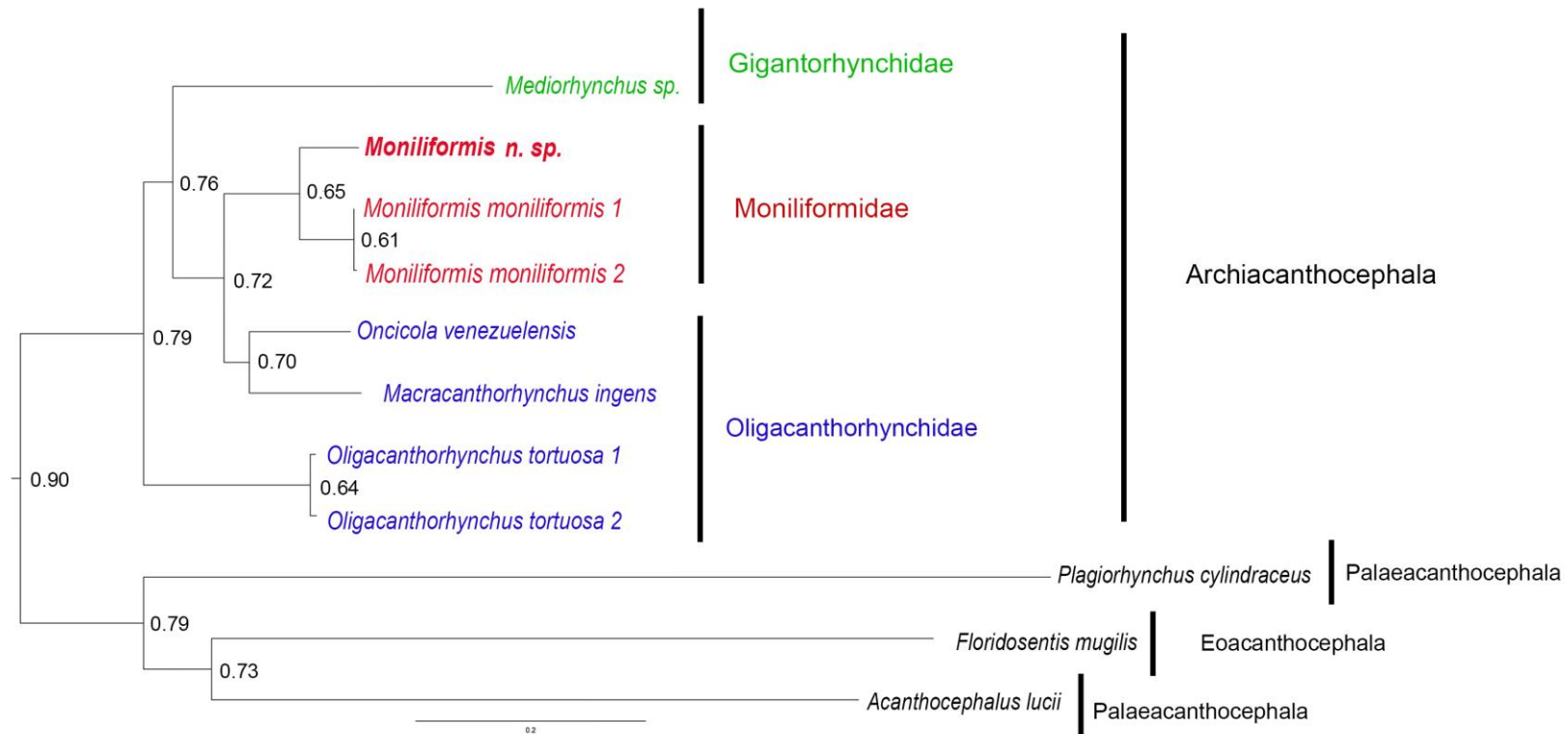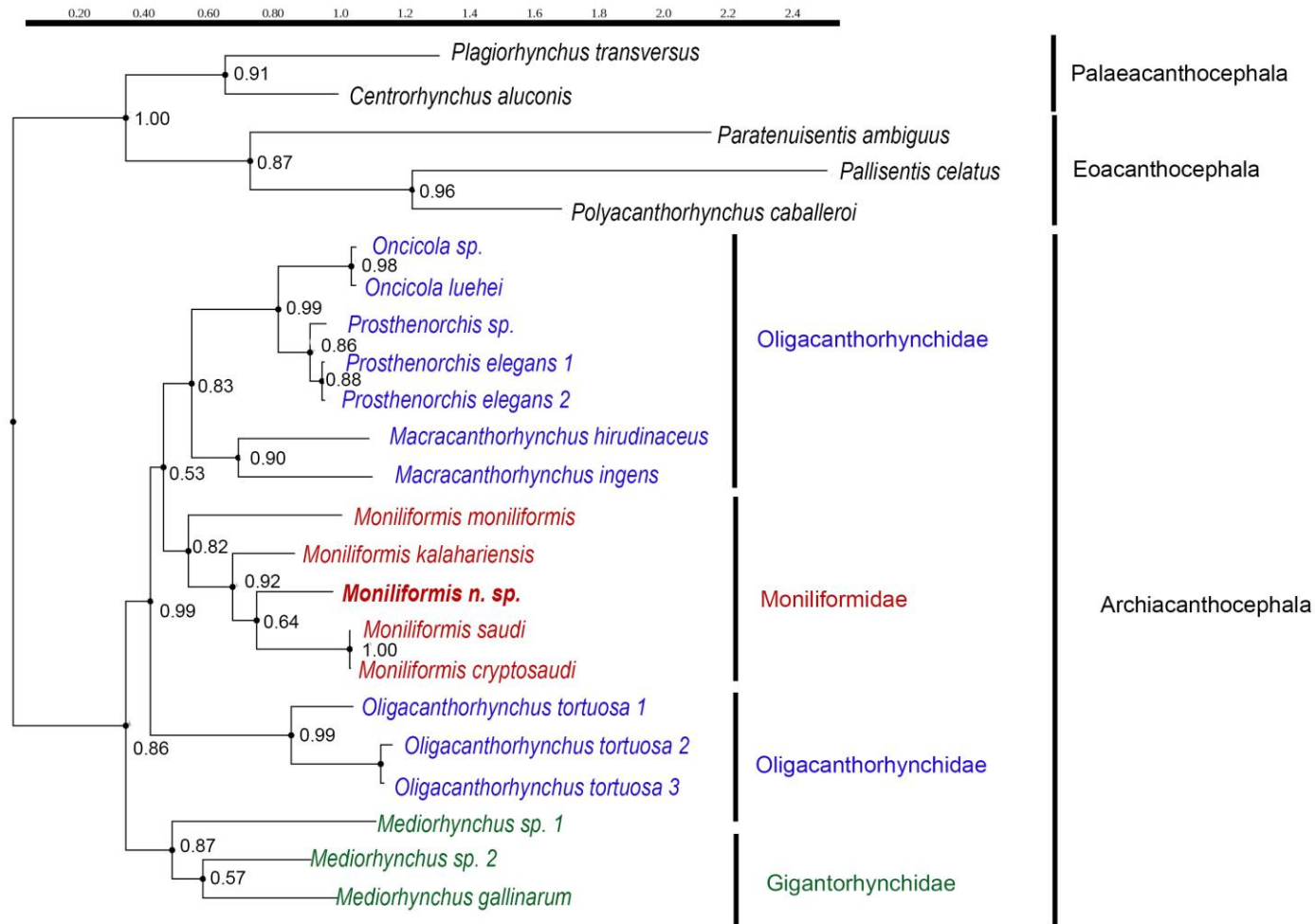
Figure 20 A. ML aLRT phylogenetic reconstruction tree of MT-CO1 gene sequences of *Moniliformis* n. sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups.
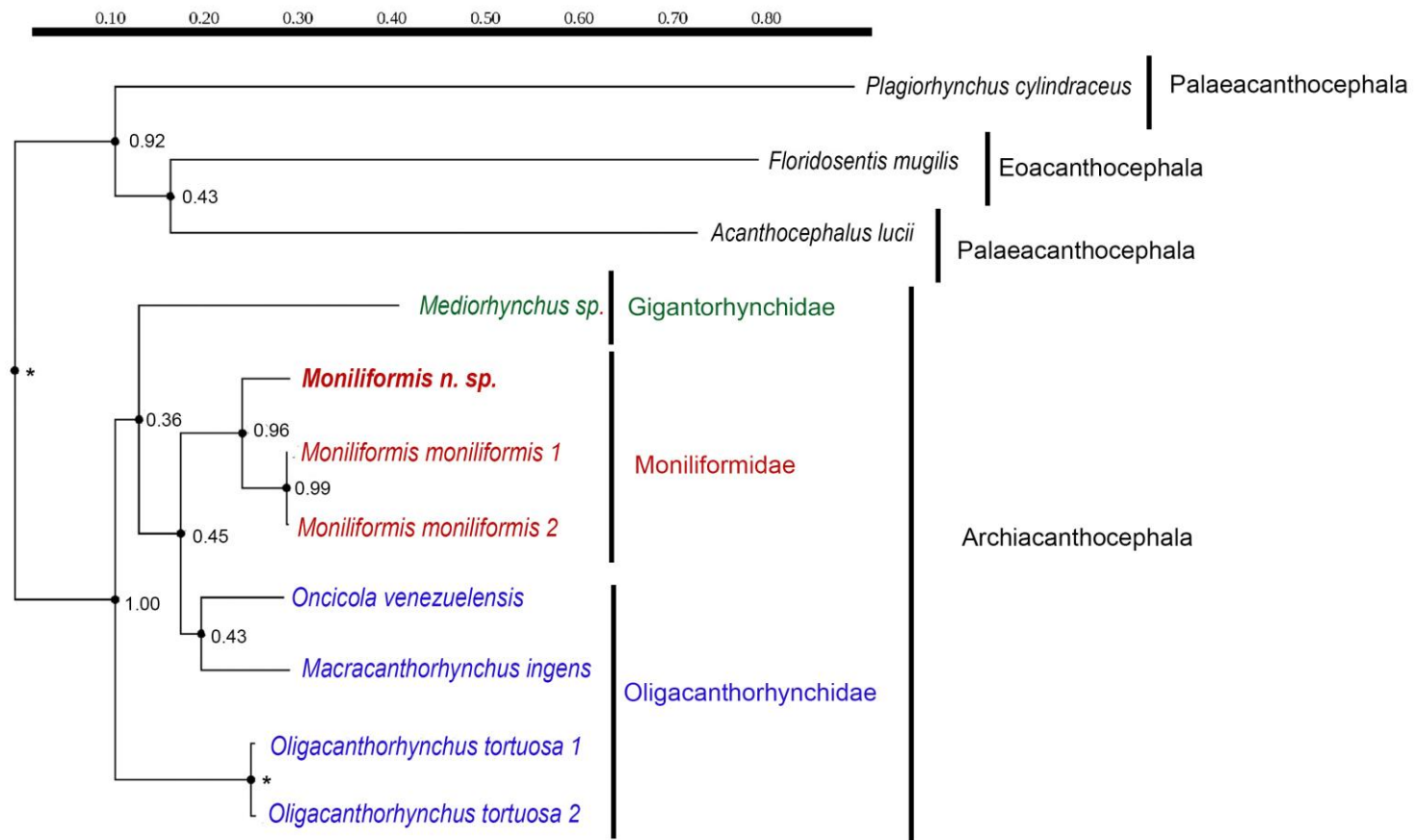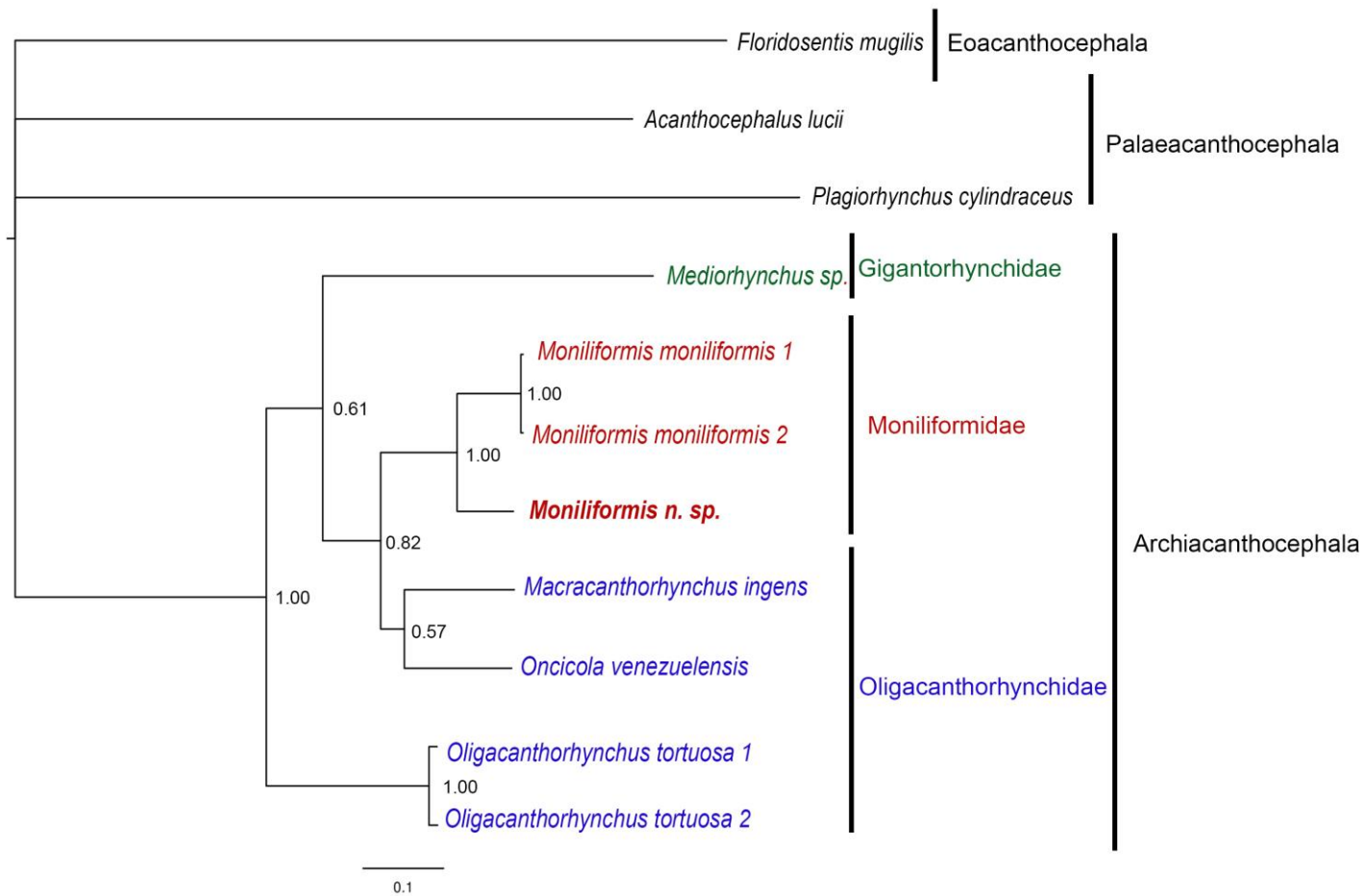
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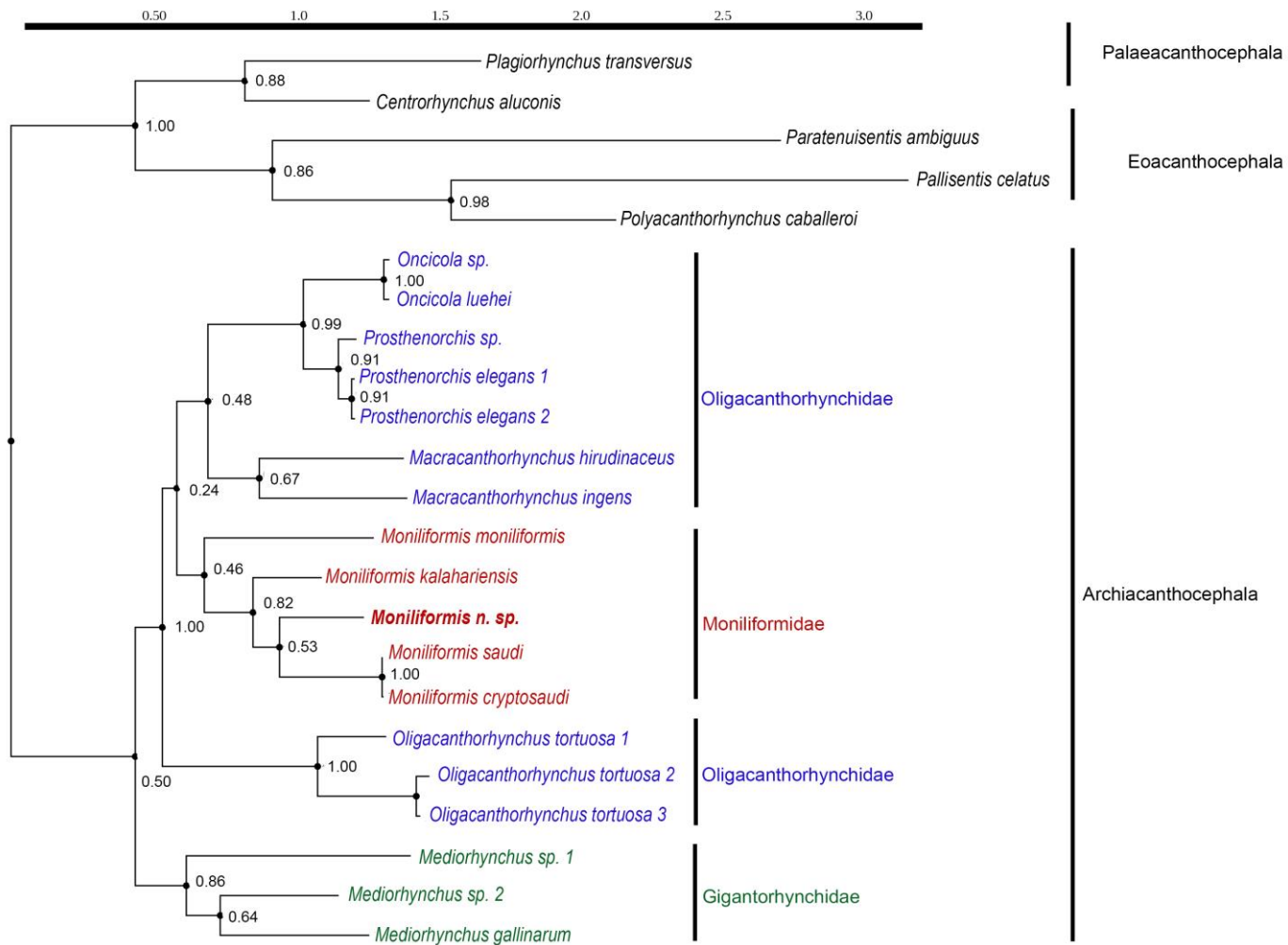
Figure 20 B. ML-BP phylogenetic reconstruction tree of MT-CO1 gene sequences of *Moniliformis* n. sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups

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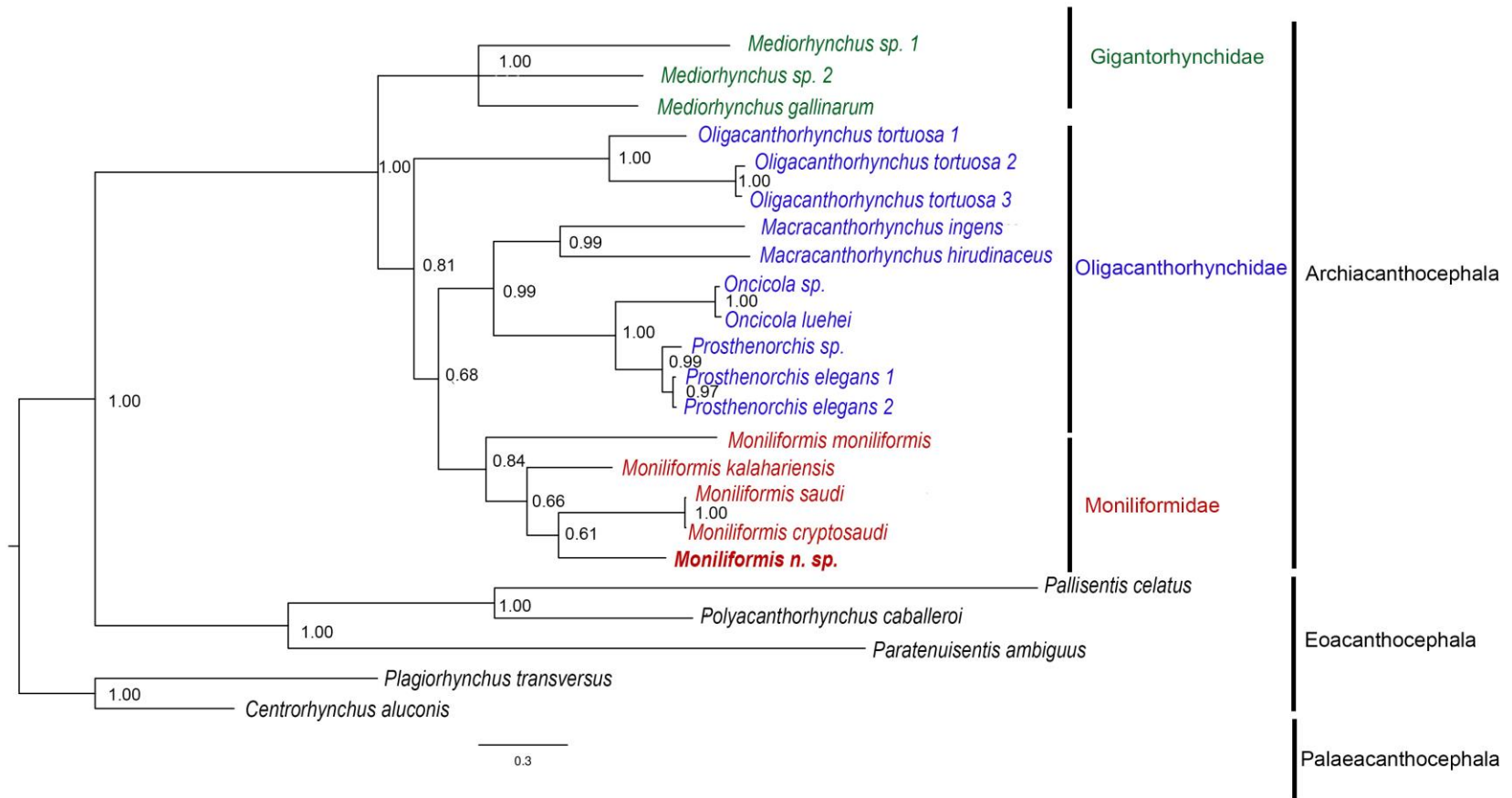
Figure 20 C. BPP phylogenetic reconstruction tree of MT-CO1 gene sequences of *Moniliformis* n. sp. in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups.

The ML genetic distance between the families Moniliformidae and Gigantorhynchidae ranged from 0.472 between *Monilformis moniliformis* and *Mediorhynchus gallinarum* to 0.376 between *Moniliformis kalahariensis* and *Mediorhynchus* sp. 2 (mean= 0.419); Moniliformidae and Oligacanthorhynchidae ranged from 0.454 *Oligacanthorhynchus tortuosa* 2 and *Moniliformis moniliformis* to 0.323 *Moniliformis kalahariensis* and *Prosthenorchis* sp. (mean= 0.388); Gigantorhynchidae and Oligacanthorhynchidae ranged 0.542 to 0.367 (mean= 0.437) (Table 3).

Analysis of ML- distance between species within the each genera of archiancthocephalans showed the following genetic distances: *Mediorhynchus* ranged from 0.382 between *Mediorhynchus* sp. 1 and *Mediorhynchus* sp. 2 to 0.320 *Mediorhynchus* sp. 2 and *M. gallinarum* (mean= 0.358); *Macracanthorhynchus* 0.370 between the *M. ingens* and *M. hirudinaceus*; *Oncicola* 0.031 between *Oncicola* sp. and *O. luehei*; *Prosthenorchis* ranged from 0.088 between *Prosthenorchis* sp. and *P. elegans* to 0.016 between the two species of *P. elegans* (mean= 0.06); Oligacanthorhynchus ranged from 0.269 *O. tortuosa* 2 and *O. tortuosa* 1 to 0.042 *O. tortuosa* 2 and *O. tortuosa* 3 (mean= 0.190). Among the sequences of *Moniliformis* species ranged from 0.368 between *M. moniliformis* and *Moniliformis* n. sp to 0.003 between *Moniliformis cryptosaudi* and *Moniliformis saudi* (mean= 0.267). The ML genetic distance of the new species *Moniliformis* n. sp. and the other species of *Moniliformis* ranged from 0.368 between the new species and *M. moniliformis* to 0.243 with *M. kalahariensis* (mean= 0.284). When we analyze de ML- distance of our new species and the two species from Middle East (Saudi Arabia and Iraq) were 0.254 and 0.273, respectively (Table 3).

Table 3. Maximum likelihood genetic p-distance over MT-CO1 gene sequence between representatives of the Acanthocephala.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*Paratenuisentis ambiguus*																							
2	*Pallisentis celatus*	0.601																						
3	*Polyacanthorhynchus caballeroi*	0.614	0.512																					
4	*Plagiorhynchus transversus*	0.689	0.657	0.606																				
5	*Centrorhynchus aluconis*	0.690	0.635	0.533	0.376																			
6	*Mediorhynchus* sp.1	0.764	0.836	0.695	0.649	0.618																		
7	*Mediorhynchus* sp. 2	0.713	0.745	0.633	0.535	0.564	0.382																	
8	*Mediorhynchus gallinarum*	0.776	0.772	0.684	0.563	0.615	0.372	0.320																
9	*Moniliformis moniliformis* 1	0.735	0.845	0.674	0.507	0.581	0.457	0.422	0.472															
10	***Moniliformis* n. sp.**	0.621	0.702	0.542	0.515	0.536	0.405	0.393	0.398	0.368														
11	*Moniliformis kalahariensis*	0.698	0.795	0.584	0.491	0.535	0.392	0.376	0.383	0.355	0.243													
12	*Moniliformis saudi*	0.682	0.757	0.615	0.528	0.576	0.424	0.415	0.412	0.335	0.254	0.254												
13	*Moniliformis cryptosaudi*	0.740	0.793	0.678	0.576	0.624	0.442	0.455	0.438	0.351	0.273	0.260	0.003											
14	*Macracanthorhynchus ingens*	0.710	0.821	0.667	0.636	0.609	0.471	0.394	0.465	0.420	0.416	0.365	0.367	0.383										
15	*Macracanthorhynchus hirudinaceus*	0.674	0.652	0.633	0.548	0.570	0.425	0.464	0.434	0.392	0.357	0.358	0.397	0.400	0.370									
16	*Oncicola* sp.	0.777	0.723	0.642	0.556	0.551	0.442	0.420	0.367	0.414	0.375	0.345	0.373	0.374	0.383	0.364								
17	*Oncicola luehei*	0.733	0.693	0.615	0.548	0.562	0.458	0.407	0.372	0.410	0.333	0.338	0.365	0.367	0.374	0.317	0.031							
18	*Prosthenorchis* sp.	0.728	0.688	0.598	0.546	0.558	0.466	0.425	0.394	0.402	0.388	0.329	0.370	0.391	0.355	0.385	0.233	0.232						
19	*Prosthenorchis elegans* 1	0.766	0.744	0.629	0.569	0.557	0.461	0.417	0.379	0.401	0.384	0.345	0.392	0.397	0.354	0.370	0.214	0.220	0.088					
20	*Prosthenorchis elegans* 2	0.782	0.753	0.626	0.566	0.557	0.458	0.415	0.372	0.398	0.383	0.341	0.385	0.390	0.359	0.368	0.219	0.217	0.088	0.016				
21	*Oligacanthorhynchus tortuosa* 1	0.761	0.790	0.669	0.668	0.586	0.521	0.439	0.445	0.445	0.413	0.383	0.397	0.401	0.453	0.424	0.429	0.444	0.412	0.410	0.406			
22	*Oligacanthorhynchus tortuosa* 2	0.750	0.795	0.712	0.685	0.574	0.542	0.452	0.415	0.454	0.393	0.425	0.408	0.422	0.432	0.448	0.444	0.449	0.457	0.435	0.437	0.270		
23	*Oligacanthorhynchus tortuosa* 3	0.733	0.784	0.681	0.650	0.563	0.504	0.453	0.431	0.435	0.364	0.413	0.401	0.407	0.433	0.434	0.420	0.437	0.411	0.421	0.417	0.258	0.042	

6.4 Discussion

The genus *Moniliformis* was proposed by Travassos (1915) which included the species *Moniliformis moniliformis* (syn. *Echinorhynchus moniliformis*) (Bremser, 1811) as type species. Travassos (1917) revised the family Gigantorhynchidae and allocated the genus *Moniliformis* to the subfamily Gigantorhynchinae with two species: *Moniliformis moniliformis* and *Moniliformis cestodiformis.* Southwell and Macfie (1925) considered valid the family Moniliformidae described by Van Cleave (1924) and included the genus *Moniliformis* with the two valid species considered by Travassos (1917). Van Cleave (1953) and Yamaguti (1963) agreed with Southwell and Macfie and both considered the genus *Moniliformis* within the family Moniliformidae. Later, Schmidt (1972) revised the class Archiacanthocephala and created a new order, Moniliformida. Thereafter, Amin (2013) updated the classification of Acanthocephala and considered valid the order Moniliformida with a single family Moniliformidae that has three genera: *Australiformis* Schmidt et Edmonds, 1989, *Promoniliformis* Dollfus et Golvan, 1963, and *Moniliformis* Travassos, 1915, the last one having 18 valid species. Recently, Amin et al. (2016) reviewed the genus *Moniliformis* and recognized 14 valid species describing a 15th species: *Moniliformis saudi* from the hedgehog *Paraechinus aethiopicus* Ehrenberg, 1832 in Saudi Arabia. Later, Martins et al. (2017) added another new species to the genus: *Moniliformis amini* from the sigmodontine rodent *Abrothrix olivaceus* (Waterhouse, 1837) in Argentina. Finally, Amin et al. (2019) described another new species from the long-eared hedgehog *Hemiechinus auritus* (Gmelin, 1770) in Iraq. To date, the genus *Moniliformis* comprises 17 species and is characterized by the presence of cylindrical proboscis with numerous and small rootless hook; body with pseudo-segmentation; long and filiform leminisci with nucleus; ellipsoid's testes and cement gland in number of 8 with spherical shape (Travassos, 1917; Southwell and Macfie, 1925; Van Cleave, 1923, 1953; Yamaguti, 1963). Species of *Moniliformis* are parasites of mammals and occasionally birds (Yamaguti, 1963; Amin et al., 2016).

The new species found in the rodent *Necromys lasiurus* were identified as belonging to *Moniliformis* due to the presence of cylindrical proboscis with 12 row of 9 to 10 small rootless hooks, double walled receptacle, ellipsoid's testes, eight grouped spherical cement glands, and female with terminal gonopore.

Moniliformis n. sp. was distinguished from *M. gracilis*, *M. tarsi*, *M. convolutus*, *M. kalahariensis*, *M. cestodiformis*, *M. saudi*, *M. monoechinus, M. cryptosaudi*, and *M. echinosorex* by the number of rows and hooks per row, the host because these moniliformid species do not parasite rodents, and the geographic distribution.

According to Amin et al. (2016) and Martins et al. (2017), only eight species have been recorded in rodents, mainly in the family Muridae, in different geographic regions of the world. The main characteristics that distinguished the new species from moniliformid species of rodents such as *M. travassosi*, *M. clarki*, *M. spiralis*, *M. aegyptiacus*, and *M. siciliensis* was the number of rows and hooks per row. Although, the range of the number of rows and hooks per row described in *M. acomysi*, *M. moniliformis*, and *M. amini* are similar to the new species, the size of the proboscis and the eggs distinguished the new species from *M. moniliformis and M. amini*. Nevertheless, *Moniliformis* n. sp. was distinguished from *M. acomysi* by the size of the body, host, and geographic distribution, since this species occur in *Acomys cahirinus* Geoffroy, 1803 in Cairo, Egypt, Africa.

In spite of a limited number of GenBank sequences available, we inferred the phylogenetic relationships of representatives of the genus *Moniliformis* based on the 28S rRNA and MT-CO1 genes sequences. Our molecular phylogenetic analyses, suggested that *Moniliformis* n. sp. nested within other species of the genus *Moniliformis*, especially with the sequences of *M. saudi* and *M. cryptosaudi*, forming a monophyletic group, and agreed with our conclusion based on morphology. Furthermore, our phylogenetic analyses of the class Archiacanthocephala genera agreed with previous studies recovering the family Moniliformidae as sister to Oligacanthorhynchidae, although with low to moderate support (García-Varela and Pérez-Ponce de León, 2015; Amin et al., 2016, Amin et al., 2019). In addition, intraspecific ML- distances between the *Moniliformis* n. sp. sequence and the other sequences of *Moniliformis*

ranged of 0.243 to 0.368 suggesting that it may represent another taxon when compared to the intraspecific genetic distances of species within other archiacanthocephalan genera.

The records for Acanthocephala in wild rodents are scarce and *Moniliformis* n. sp. is the first moniliformid species to be described from wild a rodent in Brazil. Our studies, contributed with morphological and molecular data of this new species, adding more information on species of the genus *Moniliformis* and their relationships.

Acknowledgments

7 GENERAL DISCUSSION

The helminths of the phylum Acanthocephala have been described in Brazil in different vertebrate hosts and distinct geographic regions, mainly in aquatic vetebrates as fishes species. However, studies on acanthocephalans from Brazilian mammals need revision of some taxa due to incomplete taxonomic information (Vieira et al., 2008; Muniz et al., 2009). There is also a lack of data regarding molecular and ecological studies (Amin et al, 2013, 2016, 2019; Santos et al., 2017).

The integrative taxonomy has been used to delimit and identify different taxa using together disciplines as morphology, genetics and molecular phylogeny (Dayrat, 2005). Nowadays, acanthocephalans species have been described using the integrative taxonomy including mainly morphologic and genetic approaches (Amin, 2013, 2016, 2019; García-Varela et al., 2005; Hernández-Orts et al., 2017; Liang et al., 2017; Malyarchuk et al., 2014).

Thus, the present study included the integrative taxonomy of acanthocephalans from Brazilian wild mammals from the helminthological collection of the Laboratory of Biology and Parasitology of Wild Reservoirs Mammals of Oswaldo Cruz Foundation (IOC/Fiocruz) using morphological, molecular and ecological traits.

At first, the variation in the prevalence and abundance of acanthocephalans in brown-nosed coati *Nasua nasua* and crab-eating fox *Cerdocyon thous* in the Brazilian Pantanal wetland was analysed. The studies of ecology of Acanthocephala have focused mainly on aquatic arthropods and aquatic vertebrates (Liat and Pike, 1980; Amin, 1984; Sinisalo et al., 2004; Kennedy, 2006; Steinauer et al., 2006; Franceschi et al., 2008; Amin et al., 2008; Caddigan et al., 2014; Amin, 2016), with limited research on the ecology of acanthocephalans of terrestrial mammals (Kennedy, 2006). Our results indicated that prevalence, mean abundance and mean intensity of acanthocephalan eggs did not differ between crab-eating foxes and brown-nosed coatis. In crab-eating foxes, the exposure rates to the parasite infection are similar between sexes, which resulted in nearly equivalent parasite profiles. Bianchi et al. (2014) and Olifiers et al. (2010) discussed that male and female crab-eating foxes are monomorphic in body size and the behavioral, spatial and

foraging ecology are similar and this could explain the equivalent exposure rates of prevalence, mean intensity and mean abundance of acanthocephalan eggs found in both hosts. On the other hand, adult female and male coatis are behaviorally and spatially segregated during most of the year, with males being usually solitary, except in the breeding season (Bianchi et al., 2014). Adult males are also larger than females and engage in agonistic behaviors during the reproductive season (Olifiers, 2010). Consequently, intersexual differences in prevalence, intensity and/or abundance of parasites were expected, especially during the breeding season, due to different consumption rates of food items, and the decreased health condition. In the brown-nosed coatis, the prevalence in males and females did not differ but was higher in juveniles, which may be related to acquired immunity with age (Hudson and Dobson, 1995). Further, health and immune system could influence the parasite load because they could be affected by the age and gender of the host. However, in crab-eating foxes the results were opposite showing adults with more acanthocephalan eggs than juveniles. It was expected because adults have more time to accumulate parasites than younger animals, and can be related the parasite loads with host age or age-associated body size (Anderson and Gordon, 1982; Anderson and May, 1991; Hudson and Dobson, 1995; McCormick and Nickol, 2004)

Prevalence of acanthocephalans was higher during the wet season for both host species and all the best-fitting models had the variable "season" or "maximum temperature". This availability may reflect an increased abundance in intermediate hosts and changes in exposure rates. Although the intermediate hosts of the acanthocephalans studied here are unknown in the Pantanal, arthropods are more abundant in the warmer wet season (Santos Filho et al., 2008). Both host species may have higher consumption rates of these potential intermediate hosts during the wet season.

The other results included the study of three acanthocephalan species in different mammal's species from which two were new acanthocephalan's species. The first species described belong to the genus *Pachysentis* found in a carnivore, the brown-nosed coati. The type host of species of *Pachysentis* are primates and carnivores with geographic distribution restricted to Africa and

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North, Central and South America (Meyer, 1931; Van Cleave, 1953; Golvan, 1957; Machado-Filho, 1950; García-Prieto et al., 2012; Vieira et al., 2008; Correa et al., 2016; Muniz-Pereira et al., 2016). The genus *Pachysentis* with 10 species have been reported parasitizing mammals in Africa and the American continent (Meyer, 1931, Van Cleave, 1953, Golvan, 1957, Machado-Filho, 1950, García-Prieto et al. 2012; Vieira et al, 2008, Correa et al., 2016, Muniz-Pereira et al., 2016). Five of these species were reported in Brazil, and this was the first report of the genus in the brown-nosed coati (*Nasua nasua*). *Pachysentis* n. sp. was described by light and scanning electron microscopy. The number of hooks on the proboscis and the size of the testes were considered the best character for identifying and distinguishing species of the genus (Machado-Filho, 1950). The new species of *Pachysentis* is distinguished from the other species of the genus by the number of the hooks, the presence of barbs on the hooks, and the arrangement of the cement glands. I had the opportunity to examine specimens of *P. procubens*, *P. canicola* and *P. ehrenberg* in the Museum für Naturkunde, Berlin, and *P. gethi*, *P. rugosus*, *P. procyonis*, *P. septemserialis*, and *P. lenti* from CHIOC. The re-examine of these specimens resulted in new information of morphology of two species, *P. septemserialis* and *P. ehrenbergi* and their status in the genus. A dichotomous key was provided with 10 species considering *P. septemserialis* as synonym of *P. lenti*.

The third chapter included the study of *Giganthorhynchus echinodiscus* found in the giant anteater *Myrmecophaga tridactyla* which was redescribed due to the scarce taxonomic information. The genus *Gigantorhynchus* comprises six valid species parasites of anteaters, with two of them reported from Brazil. *Gigantorhynchus echinodiscus* reported infecting anteaters, *M. tridactyla, Tamandua tetradactyla* and *Cyclopes didactylus* (Travassos, 1917, Strong et al., 1926, Machado Filho, 1941). Amato et al. (2014) reported cystacanths of *G. echinodiscus* infecting termites as intermediate hosts. These records included descriptions based on morphological characteristics (Travassos, 1917, Machado Filho, 1941), and there was no genetic data available for the genus in public databases. Our results with molecular phylogenetic analysis showed *G. echinodiscus* forming a well-supported monophyletic group with *Mediorhynchus*

sp., which was congruent with morphological studies that allocate both genera within the family Gigantorhynchidae (Amin, 2013). The 28S rRNA gene study provided the first DNA sequence and the first phylogenetic analyses for the genus *Gigantorhynchus* that contribute to better understanding the relationship between tha acanthocephalans, especially archiacanthocephala's species.

The third species described is also a new species parasitazing the wild rodent, hairy-tailed bolo mouse *Necromys lasiurus* that belong of the genus *Moniliformis.* The genus *Moniliformis* has 17 species, which parasitize mammals and birds in the world (Amin, 2013, 2016, 2019, Martins et al., 2017). In Brazil, two species have been reported parasitizing mammals (Travassos, 1917, Machado Filho 1946; Gibson & McCarthy 1987; Tietz Marques and Scroferneker, 2003; De Araújo et al. 2014; Santos and Gibson, 2015; Simões et al., 2016). The new species of *Moniliformis* now described is distinguished from other moniliformid species by the number of rows and the number of the hooks per rows; the size of the proboscis; the size of the eggs. New molecular phylogenies inferred from partial 28S rRNA and partial mitochondrial cytochrome c oxidase subunit I gene (MT-CO1) showed *Moniliformis* n. sp. forming a well-supported monophyletic group with other sequences of *Moniliformis*. This genetic data agrees with the morphological studies, allocating the new species within the genus and the family Moniliformidae (Amin et al., 2016, Martins et al., 2017).

Finally, the present study contributed with the description of two new species, and suggested that the Brazilian acanthocephalan's mammals have underestimated biodiversity. Thus, more studies are needed, particularly with other mammal hosts species. In addition, it was performed an integrative taxonomy of acanthocephalan's species using morphologic, molecular and ecological data, expanding the geographic and host distribution of these helminths in carnivores, rodents and anteaters. This work contributed to a better understanding of the diversity and distribution of Acanthocephala species in Brazil, emphasizing the importance of integrative taxonomic studies to clarify their taxonomy.

8 CONCLUSIONS

• Factors such as temperature, seasonality, host gender and age influenced the abundance and prevalence of infection of acathocephalans from two carnivores (brown-nosed coatis and crab-eating fox) in the Panatanal weltland.

• Three acanthocephalan species were studied with two representing new species from different wild mammals and geographic distribution;

• A new species of *Pachysentis* (Archiacanthocephala: Oligacanthorhynchidae) was described from brown-nosed coati *Nasua nasua* in the Pantanal weltlands of the state of Mato Grosso do Sul was described based on morphological characteristcis by ML and SEM and adding a review of the genus;

• The identification and re-description of *Gigantorhynchus echinodiscus* (Archiacanthocephala: Gigantorhynchidae) from the giant anteater *Myrmecophoga tridactyla* in the Cerrado of the state of São Paulo provided details on the morphological structures, molecular and phylogenetic information with 28S rRNA gene that showed *G. echinodischus* forming a monophyletic group which contributes for elucidate the relationship between the genera in the family Gigantorhynchidae;

• The description of new species of *Moniliformis* (Archiacanthocephala: Moniliformidae) from a wild rodent, hairly-tailed bolo mouse (*Necromys lasiurus*), provided morphological characteristics, and molecular phylogenetic information with 28S rRNA gene and MT-CO1 gene, suggesting another taxon, and contributing with more information of the genus *Moniliformis* and their relationship.

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10 APPENDIX

10.1 Chapter 1

Variation in the prevalence and abundance of acanthocephalans in brown-nosed coatis *Nasua nasua* and crab-eating foxes *Cerdocyon thous* in the Brazilian Pantanal

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Abstract

Host infection by parasites is influenced by an array of factors, including host and environmental features. We investigated the relationship between host sex, body size and age, as well as seasonality on infection patterns by acanthocephalan in coatis (Procyonidae: *Nasua nasua*) and in crab-eating foxes (Canidae: *Cerdocyon thous*) from the Brazilian Pantanal wetlands. Between 2006 and 2009, we collected faecal samples from these hosts and analyzed for the presence of acanthocephalan eggs. Prevalence, abundance and intensity of eggs of acanthocephalans were calculated. Egg abundance was analyzed using generalized linear models (GLM) with a negative binomial distribution and models were compared by Akaike criteria to verify the effect of biotic and abiotic factors. Prevalence of acanthocephalans was higher in the wet season in both host species but did not differ between host sexes; however, adult crab-eating foxes showed higher prevalence of acanthocephalan eggs than juveniles. In contrast, prevalence of acanthocephalan eggs found in coatis was higher in coati juveniles than in adults. Host age, season and maximum temperature were the top predictors of abundance of acanthocephalan eggs in crab-eating foxes whereas season and host sex were predictors of egg abundance in coatis. The importance of seasonality for abundance of acanthocephalan was clear for both host species. The influence of host-related attributes, however, varied by host species, with host gender and host age being important factors associated with prevalence and parasite loads.

Keywords: Acanthocephala, Carnivora, disease ecology, helminth, Pantanal.

Variação na prevalência e na abundância do parasitismo de acantócefalos em dois carnívoros silvestres do Pantanal brasileiro

Resumo

A infecção de hospedeiro por parasitos é influenciada por uma série de fatores, incluindo características do hospedeiro e ambientais. Nós investigamos a relação entre sexo do hospedeiro, tamanho corporal e idade, bem como sazonalidade nos padrões de infecção por acantócefalos em coatis (Procyonidae: *Nasua nasua*) e em cachorro-do-mato (Canidae: *Cerdocyon thous*) do Pantanal brasileiro e quais fatores explicaram melhor a prevalência e a intensidade desses parasitos. Entre 2006 e 2009, coletamos amostras fecais desses hospedeiros e analisamos a presença de ovos de acantócefalos. Prevalência, abundância e intensidade de ovos de acantócefalos foram calculados. A abundância de ovos foi analisada utilizando modelos lineares generalizados (GLM) com distribuição binomial negativa e os modelos foram comparados pelo critério de *Akaike* para verificar o efeito de fatores bióticos e abióticos. A prevalência de acantócefalos foi maior na estação úmida em ambas as espécies de hospedeiros, mas não diferiu entre os sexos do hospedeiro; no entanto, os cachorros-do-mato adultos apresentaram maior prevalência de ovos de acantócefalos do que em juvenis. Em contraste, a prevalência de ovos de acantócefalos encontrados em coatis foi maior em juvenis do que em adultos. A idade do

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hospedeiro, a estação e a temperatura máxima foram os preditores de abundância de ovos de acantocéfalos em cachorro-do-mato, enquanto a estação e o sexo do hospedeiro foram preditores da abundância dos ovos do parasito em coatis. A importância da sazonalidade para a abundância do acantocéfalo foi clara para ambas as espécies hospedeiras. A influência dos atributos relacionados ao hospedeiro, no entanto, variou entre as espécies de hospedeiros, sendo o sexo e idade do hospedeiro fatores importantes associados à prevalência e às cargas parasitárias.

Palavras-chave: Acanthocephala, Carnívora, ecologia de doença, helminto, Pantanal.

1. Introduction

Helminth parasites show a variety of transmission patterns determined by their life cycle characteristics and ecological requirements. As a result, their prevalence and abundance has been correlated with both life history characteristics of the host as well as environmental factors that act on helminth development (Mas-Coma et al., 2008). While such correlations are now well-recognized for many parasitic taxa, the relative importance these biotic and abitoc factors in explaining variability in the timing of infection is often not fully understood.

Seasonal variation in temperature and humidity and host features such as feeding habits, habitat preference, age, gender and body size can regulate the host-parasitism dynamic and are often considered in ecological studies of many parasites (Behnke et al., 2001; Ferrari, 2005; Krasnov et al., 2005; Simões et al., 2014). Such factors can determine the contact rates, and thereby influencing parasite population dynamics, parasite spatial distribution, and the risk of host infection (Bush et al., 2001; Altizer et al., 2006).

Among mammals, males tend to have higher abundance, prevalence and parasite species richness than females (Poulin, 1996; Schalk and Forbes, 1997; Soliman et al., 2001; Rossin and Malizia, 2002). These trends have been related to sex-specific host behaviors, as well as distinct androgen levels, body mass differences, and higher levels of physiological stress (Brown et al., 1994; Arneberg et al., 1998; Moore and Wilson, 2002; Morand et al., 2004; Krasnov et al., 2011). Likewise, older hosts may have higher parasite loads due to the more extensive opportunity for exposure to the parasite throughout their lives (Anderson and Gordon, 1982; Anderson and May, 1991; Cooper et al., 2012; Hudson et al., 2002).

Ecological factors associated with parasitism by endoparasites have primarily focused on nematodes of mammals (e.g. Brouat et al., 2007; Simões et al., 2012; Cardoso et al., 2016; Spickett et al., 2017). Few studies have addressed the Phylum Acanthocephala. Acathocephalans are a group of intestinal parasites with wide geographic distribution and approximately 1,300 species (Amin, 2013). Adult parasites attached to the wall of the intestine in the definitive host, causing various pathological conditions such as chronic enteritis with ulcerative lesions (Dunn, 1963; Müller et al., 2010). They typically display a two-host, indirect life cycle involving a variety of arthropods (insects and crustaceans) as intermediate hosts and vertebrates (fish, amphibians, reptiles, birds and mammals) as definitive hosts (Read, 1974; Crompton and Nickol, 1985).

The ecology of the Acanthocephala has mainly been studied in aquatic arthropods and aquatic vertebrates (Liat and Pike, 1980; Amin, 1984; Sinisalo et al., 2004; Kennedy, 2006; Steinauer et al., 2006; Franceschi et al., 2008; Amin et al., 2008; Caddigan et al., 2014; Amin, 2016), with limited research on the ecology of acanthocephalans of terrestrial mammals (Kennedy, 2006). For example, to our knowledge there have been no ecological studies of acanthocephalans from mammalian wildlife in Brazil. The aim of this study was to examine how biotic and abiotic features influence parasitological parameters of Acanthocephala found in brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) in the Brazilian Pantanal.

The crab-eating fox *Cerdocyon thous* (Linnaeus, 1766) is a monogamous, sexually monomorphic canid with a social structure composed of two to five individuals, usually a breeding pair with pups and sometimes offspring from previous years (Courtenay and Maffei, 2004; Bianchi et al., 2016). In contrast, the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) is a polygynous, sexually dimorphic species in which adult males are larger than females (Olifiers, 2010). Adult females and juvenile form groups of several individuals, but adults males are typically solitary outside of the reproductive season (Gompper and Decker, 1998; Bianchi et al., 2014). After the breeding season, pregnant females give birth in a nest, usually constructed on a tree, since this species is scansorial (Olifiers et al., 2009). Both species have generalist omnivorous diets, consuming fruits, gastropods, arthropods such as arachnids, insects, myriapods, as well as small vertebrates (Bianchi et al., 2014; Olmos, 1993; Pedó et al., 2006).

Although both coatis and crab-eating foxes have generalist diets (Bianchi et al., 2014) and inhabit similar habitats, their distinct reproductive behavioral and sex-related morphologic features may result in different infection patterns. As a consequence, parasite load is expected to be higher in coati males than females, but not to differ by gender for the monomorphic crab-eating foxes. On the other hand, patterns of parasitism should also vary with abiotic factors in habitats with strong seasonality. For example, the Brazilian Pantanal, where both coatis and crab-eating foxes are sympatric, presents two makedly different seasons, with higher temperature and humidity during the wet season that can favor the life cycle of parasites and their intermediate hosts (e.g., for acanthocephalans: Kennedy, 2006; Amin, 1980). If abiotic factors are more important than factors intrinsic to the host in mediating the parasite-host dynamic, we expect

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the two parasite-host dyads to show similar quantitative relationships despite the differing ecologies of the hosts.

2. Material and Methods

2.1. Study area

The Pantanal biome is the largest wetland in the world and harbors a high density and diversity of vertebrates, particularly mammals (Tomás et al., 2010; Alho et al., 2011; Alho and Sabino, 2011). Field work was conducted at Nhumirim Ranch (18°59'S, 56°39'W), a 4,400 ha research station of the Brazilian Agricultural Research Corporation (Embrapa) in the Nhecolândia subregion of the Pantanal State of Mato Grosso do Sul, Brazil. The study area is characterized by sandy soil with mosaic vegetation of semi-deciduous forest with open grassy areas and seasonally flooded fields (Rodela, 2006). The climate is tropical with two distinct seasons: wet season (October to March) and dry season (April to September).

2.2. Capture procedures

From 2006 to 2009 we captured/recaptured *Cerdocyon thous* and *Nasua nasua* which were the subject of a broader research program conducted by Embrapa/Pantanal and the Oswaldo Cruz Foundation (FIOCRUZ-RJ). As part of this research, we collected feacal samples from known individuals for gastro-intestinal parasite diagnosis. Animals were captured every 3 to 4 months using wire box traps (1 m × 0.40 m × 0.50 m) placed in a trapping grid of 7.2 Km², but traps were also occasionally placed outside the grid. Traps were baited with bacon, set late in the afternoon and checked in the morning. The captured animals were anesthetized, tagged with numbered colored tag (Nasco Rototag®) and/or subcutaneous transponder (AnimalTag®), measured, weighed and sexed. Tooth eruption, condition and wear were also recorded to age individuals (Olifiers et al., 2010). Feacal samples were collected from beneath traps or via fecal loop. After sample collection, the animals were released at their capture sites. The animal capture and handling procedures were approved by the Brazilian Federal Environmental Agency (IBAMA, first license #183/2005, CGFAU/LIC; last license #11772-2) and by the University of Missouri Animal Care and Use Committee (protocol #4459).

2.3. Parasitological procedures

Feces collected from each animal (1-3 g) were stored in 15mL of 10% formalin and analyzed in the laboratory using methods for endoparasites diagnostics: flotation in sugar solution (density 1.27), sedimentation and centrifugation with formol-ether (Bowman, 1999). After sedimentation, the pellet was resuspended in 1 mL of 10% formaldehyde and a subsample of 80 µL was placed on a slide for analysis in the light microscope (Monteiro et al., 2007). Slides from the sugar flotation and sedimentation techniques were analyzed at 100x and 400x magnification. Eggs of acanthocephalans were photographed, measured, and compared with the morphology described according to Yamaguti (1963), Schmidt (1972), and Machado Filho

(1950). In addition, adults specimens of acanthocephalans were collected from the intestine of three crab-eating foxes and two brown-nosed coatis found dead in the study area. The adults specimens were analysed and described/identified as the *Prosthenorchis cerdocyonis* (Gomes et al., 2015; type species CHIOC 35804 a-c) and *Pachysentis* sp. (deposit pending), respectively. Because co-infection by acanthocephalan species are apparently rare (Kennedy, 2006) and the eggs found in fecal flotation were very similar in size and shape to the eggs obtained from the female acanthocephalans recovered from the dead hosts, we suggest that we are identifying and quantifying *P. cerdocyonis* from crab-eating foxes and *Pachysenti* sp. from coatis. However, since we cannot discard the possibility of co-infection by other (perhaps undescribed) acanthocephalan species parasitizing coatis and crab-eating foxes in the study area, we classified the eggs as belonged to acanthocephalans from the Class Archiacanthocephala, Order Oligacanthorhynchida, Family Oligocanthorhynchidae. The number of acanthocephalan eggs in the faecal samples was divided by the total weight of analyzed feces and used as proxy of parasite abundance. When more than one sample for the same host was obtained in the same excursion (recaptured animals), we calculated the mean number of eggs obtained for the samples analyzed for that period.

2.4. Data analyses

We calculated the prevalence as the estimated number of infected hosts divided by the total number of analyzed hosts. Abundance was estimated as the number of eggs per gram of feces found in each individual host and the intensity was the number of eggs per gram of feces found in infected hosts (Bush et al., 1997). Prevalence was compared between sexes, age and seasons using Chi-square tests ($\alpha = 0.05$) for each host species. Mean intensity and mean abundance were also compared between species using the program Quantitative Parasitology 3.0 (QP3.0; Reiczigel and Rózsa, 2005). Confidence intervals (95% CI) for prevalence were calculated using the Clopper-Pearson interval method, and for mean and median intensity as well as mean abundance by bootstrap tests (n = 2000) using QP 3.0. The level of aggregation of both acanthocephalan species on their respective hosts was quantified by calculating the negative binomial exponent, k (Wilson et al., 2002).

To analyze the effect of biotic (age, sex, body size) and abiotic factors (season, temperature and humidity) on the abundance acanthocephalan eggs (dependent variable) we created generalized linear models (GLM) with negative binomial distributions and log link in SPSS 20, as the data showed a predominantly aggregated distribution for both parasite species (see results). Before creating the models, we checked whether abiotic variables (minimum, maximum and average temperature, relative humidity and precipitation) were correlated (Pearson correlation, $\alpha = 0.05$). The final factors used to create the models were maximum temperature (MT), relative humidity (RH) and season (dry and wet season). Abiotic data was obtained

from the Instituto Nacional de Meteorologia and averaged for 30 days before the date of the fecal sample collection. Host body size (mm) was measured from the tip of the nose to the base of the tail (Olifiers, 2010). Host age was estimated based on morphometric measurements and dental condition following Olifiers et al. (2010), which allowed placement of animals into one of four age categories. We further combined classes due to small sample sizes for some age groups such that all animals were ultimately classified as juveniles ($\leq$ 2 years old) or adults (> 2 years old).

The evaluated models consisted of all possible combinations of the six independent predictors (64 models in total); five additional models having interaction terms were included after investigation of predictor vs. response variable plots revealed possible interaction between these variables. Models were compared using the Akaike Information Criterion corrected for overdispersion (QAICc) and ranked based on the difference between the best approximating model (model with the lowest QAICc) and all others in the set of candidate models (ΔQAICc). Models with differences within two units of the top model were considered competitive models with empirical support (Burnham and Anderson, 2001). The relative importance of each predictor or interaction of predictors was quantified by calculating relative variable weights, which consists of the summed Akaike weights (QAICc weights) across all the models in which the predictor occurs. Variables weights lower than 0.40 were considered indicative of relatively low variable importance.

3. Results

We analyzed 118 fecal samples from 55 crab-eating foxes (24 females and 31 males) and 72 fecal samples from 61 brown-nosed coatis (13 females and 48 males) throughout 10 field excursions (see Table 1 and 2). Prevalence of acanthocephalan eggs did not differ between crab-eating foxes (22.9%; n = 118) and brown-nosed coatis (29.2%; n = 72; Chi-square = 0.936; p = 0.333). Likewise, mean abundance (t-statistic = -0.607; p = 0.556) and mean intensity (t-statistic = -1.903; p = 0.061) did not differ between host species. Egg abundance was similarly aggregated in both hosts (acanthocephalan eggs in crab-eating foxes: k = 0.1031, Figure 1; acanthocephalan eggs in coatis: k = 0.1734, Figure 2).

3.1. Ecological analyses of acanthocephalan in crab-eating foxes (Cerdocyon thous)

Differences in prevalence between host sexes (Chi-square = 0.066, p = 0.797) or age categories were not significant (Chi-square = 1.771; p = 0.183). However, prevalence of eggs tended to be higher during the wet season (32.6%) than in the dry season (17.3%), although the difference was only marginally significant (Chi-square = 3.590, p = 0.058) and 95% CIs of intensity and abundance overlapped.

Four models were supported (ΔQAICc < 2) in the analysis of the abundance acanthocephalan eggs found in crab-eating foxes, but their individual QAICc weights were relatively low (from 0.05 to 0.13; Table 3). The top ranked

Table 1. Ecological parameters for *Prosthenorchis cerdocyonis* eggs in crab-eating foxes (*Cerdocyon thous*) sampled in the Brazilian Pantanal from 2006 to 2009.

Categories	N	Prevalence (%)	Mean Intensity	Median Intensity	Mean Abundance
All	118	22.9% (15.65-31.52)	6.0 (4.78-7.93)	4.0 (4.0-8.0)	1.37 (0.89-2.04)
Females	55	21.8% (12.46-34.45)	6.0 (4.67-7.92)	5.0 (4.0-8.0)	1.31 (0.67-2.20)
Males	63	23.8% (13.98-36.22)	6.0 (4.20-9.00)	4.0 (2.0-8.0)	1.43 (0.78-2.59)
Adults	70	27.1% (17.19-39.10)	6.84 (5.32-9.32)	7.0 (4.0-8.0)	1.86 (1.13-2.91)
Juveniles	48	16.7% (7.48-30.23)	4.0 (2.88-5.00)	4.0 (2.0-6.0)	0.67 (0.29-1.21)
Dry season	75	17.3% (9.56 - 27.82)	7.23 (5.15 - 11.00)	6.0 (3.0 - 8.0)	1.25 (0.67 - 2.29)
Wet season	43	32.6% (19.07-48.55)	4.86 (3.57-6.14)	4.0 (2.0-7.0)	1.58 (0.88-2.47)

Numbers between brackets are 95% confidence intervals; N = number of sampled hosts.

Table 2. Ecological parameters for *Pachysentis* sp. eggs in brown-nosed coatis (*Nasua nasua*) sampled in the Brazilian Pantanal from 2006 to 2009.

Categories	N	Prevalence	Mean Intensity	Median Intensity	Mean Abundance
All	72	29.2% (19.04-41.07)	3.81 (2.52-5.86)	2.0 (1.0-4.0)	1.1 (0.64-1.96)
Females	13	23.1% (5.03-53.82)	2.0 (1.00-2.67)	2.0*	0.46 (0.08-1.15)
Males	59	30.5% (19.18-43.87)	4.06 (2.61-6.44)	2.5 (1.0-4.0)	1.24 (0.68-2.22)
Adults	26	15.4% (4.35-34.87)	6.5 (3.50-10.75)	5.5*	1.0 (0.27-2.54)
Juveniles	46	37.0% (23.20-52.46)	3.18 (2.00-5.71)	2.0 (1.0-3.0)	1.17 (0.63-2.37)
Dry season	26	11.5% (2.44-30.16)	2.0 (1.00-2.67)	2.0*	0.23 (0.04-0.58)
Wet season	46	39.1% (25.08-54.63)	4.11 (2.67-6.33)	2.5(1.0-4.0)	1.61 (0.87-2.76)

Numbers between brackets are 95% confidence intervals; N = number of sampled hosts; *Confidence level cannot be reached because the sample size is small.
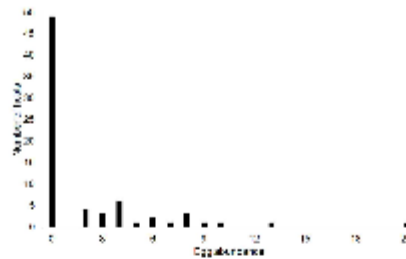
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Figure 1. Distribution of acanthocephalan egg abundance (eggs/g of feces) in crab-eating foxes (*Cerdocyon thous*) from the Brazilian Pantanal wetlands.
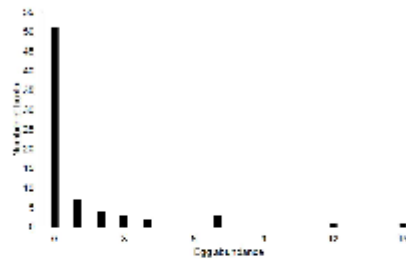

Figure 2. Distribution of acanthocephalan egg abundance (eggs/g feces) in brown-nosed coatis (*Nasua nasua*) from the Brazilian Pantanal wetlands.

model supported an interaction of season and age, followed for three models that included maximum temperature either alone or in combination with host age (Table 3). Indeed, the contributions of age (var. weight = 0.75, β = 1.08), maximum temperature (var. weight = 0.56; β = 0.197) and season (var. weight = 0.41; $β_{dry}$ = - 0.43) to variation in abundance of the acanthocephalan eggs in crab-eating foxes were higher than all other variables.

3.2. Ecological analyzes of acanthocephalan eggs in brown-nosed coatis (Nasua nasua)

Prevalence in coati males and females did not differ (Chi-square = 0.285; p = 0.594), but prevalence was higher in juveniles than in adults (Chi-square = 3.742; p = 0.053). Egg prevalence was over 3 times higher in the wet season than in the dry season (Chi-square = 6.121; p = 0.013) (Table 2). Similarly, measures of intensity and abundance were higher during the wet season and 95% CIs were non-overlapping for the means of both.

Five top models were supported (ΔQAICc < 2) for the abundance of acanthocephalan eggs in coatis, and these models collectively contained five variables: season (var. weight = 0.88, $β_{dry}$ = -1.816), sex (var. weight = 0.46; $β_{female}$ = -1.316), maximum temperature (var. weight = 0.27, β= 0.114), body size (var. weight = 0.26, β = -0.005), and relative humidity (var. weight = 0.24, β = -0.019) occurred in these most-supported models (Table 4). The variable weights for season, which occurred in all five top models, and sex (which occurred in two of the top models) were higher than 0.40, suggestive of strong support.

Table 3. Ranking of the best-fitting models describing *P. cerdocyonis* egg abundance in crab-eating foxes (*Cerdocyon thous*) in the Pantanal wetlands, Mato Grosso do Sul, Brazil from 2006 to 2009.

Model	Log(l)/c	QAICc	k	ΔQAICc	QAICc Weight
Season × Host age	-56.30	123.15	5	0.00	0.13
Host age + Max. temperature	-57.76	123.87	4	0.73	0.09
Max. temperature ×Host age	-57.82	123.99	6	0.84	0.09
Max. temperature	-59.46	125.13	3	1.98	0.05

Season = dry and wet seasons; Max. temperature = daily maximum temperature averaged for 30 days before the date of the focal sample collection. Only models with ΔQAICc ≤ 2 are shown. Akaike Information Criterion corrected for overdispersion (QAICc), Akaike weights (QAICc weights).

Table 4. Ranking of the best-fitting models describing abundance of *Pachysentis* sp. eggs in brown-nosed coati (*Nasua nasua*) in the Pantanal wetlands, Mato Grosso do Sul from 2006 to 2009.

Model	Log(l)/c	QAICc	k	ΔQAICc	QAICc Weight
Season	-42.94	92.23	3	0.00	0.13
Season + Host sex	-41.95	92.50	4	0.27	0.11
Season + Humidity	-42.44	93.48	4	1.25	0.07
Season + Body size + Host sex	-41.54	93.99	5	1.76	0.05
Season + Max. temperature	-42.73	94.06	4	1.83	0.05

Season = dry and wet seasons; Max. temperature = daily maximum temperature averaged for 30 days before the date of the focal sample collection; Humidity = daily averaged for 30 days before the date of the focal sample collection. Only models with ΔQAICc ≤ 2 are shown. Akaike Information Criterion corrected for overdispersion (QAICc), Akaike weights (QAICc weights).

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4. Discussion

In this study the overall patterns of prevalence, intensity and abundance were similar for acanthocephalans in both hosts. The samples of the present study were collected in the same study area and both definitive hosts have similar habitats and diets (Olifiers et al., 2010; Bianchi et al., 2014, 2016), which suggests these host species may have similar probabilities of contact with infected intermediate hosts. Although coatis are scansorial and therefore can climb trees, they spend most of their foraging time on the ground (Hirsch, 2009).

Prevalence of acanthocephalans in crab-eating foxes was not different between host sexes, and neither host age nor host body size appeared amongst the best-fitting models. Male and female crab-eating foxes are monomorphic in body size, and the behavioral, spatial and foraging ecology of males and females are similar (Brady, 1979; MacDonald and Courtenay, 1996; Bianchi et al., 2014; Olifiers et al., 2010). Although some studies have shown that higher androgen levels in males may lead to higher parasite intensity or prevalence (Moore and Wilson, 2002; Muehlenbein and Watts, 2010), this hypothesis does not hold for the acanthocephalans eggs found in crab-eating foxes. It seems that exposure rates to the parasite are similar between sexes and resulted in nearly equivalent parasite profiles for males and females.

In contrast to the crab-eating foxes, adult female and male coatis are behaviourally and spatially segregated during most of the year, with males usually solitary, except in the breeding season (Bianchi et al., 2014). Adult males are also larger than females and engage in agonistic behaviours during the reproductive season (Olifiers, 2010). Consequently, intersexual differences in prevalence, intensity and/or abundance of parasites are expected for this host species, especially during the breeding season, due to different testosterone levels, different consumption rates of food items, and the decreased health condition of breeding season males. Indeed, model analysis for abundance of acanthocephalan eggs in coatis indicated that host sex was an important predictor of infection; male coatis seem to be more affected by parasitism, especially during the breeding season, which may in turn favor higher parasite intensities. Olifiers et al. (2015) found similar results for *Trypanosoma evansi* infection in coatis from the same study site.

Adult crab-eating foxes had more acanthocephalan eggs than juveniles (Table 1). This result is expected, given that adults have more time to accumulate parasites than younger animals. Older hosts may have been exposed to more parasites during their lifetime, as observed in other studies in which there was a continuous increase in parasite loads with host age or age-associated body size (Anderson and Gordon, 1982; Anderson and May, 1991; Hudson and Dobson, 1995; McCormick and Nickol, 2004). However, coatis showed the opposite pattern, with prevalence (but not intensity) being higher in juveniles than in adults (Table 2). Although such result may be related to acquired

immunity with age, it is not clear why this process would occur in coatis but not in crab-eating foxes.

Prevalence of acanthocephalans was higher during the wet season for both host species (Table 1 and 2) and all the best-fitting models had the variable "season" or "maximum temperature" (Table 3 and 4). Thus, acanthocephalans from brown-nosed coatis and crab-eating foxes are likely more available to hosts during the wet season. This availability may reflect an increased abundance in intermediate hosts and changes in exposure rates. Furthermore, model analysis revealed higher parasite abundance for acanthocephalan eggs in coatis feces just after a humid month, while abundance of acanthocephalan eggs in crab-eating foxes was higher just after months with higher maximum temperature. Chubb (1982) and Kennedy (2006) showed seasonal cycles in prevalence and abundance of acanthocephalans that were correlated with temperature. Likewise, Amin et al. (2008) also suggested a seasonal pattern of acanthocephalan infection and showed that prevalence of acantocephalans may increase during the summer in freshwater fishes from Lake Malawi, due to the sexual maturity and breeding activity in the end of winter and early spring. In addition, Amin (1980, 1987) and Kennedy (2006) analyzed the ecology of intermediate hosts and showed that in warm temperatures, parasite development increases as cystacanths (the infective stage to the definitive host) in the intermediate host; a greater proportion of gravid female worms are found in the definitive host during the summer; and the definitive host consumed more infected intermediate host in the summer, resulting in higher transmission rates.

Although the intermediate hosts of the acanthocephalans studied here are unknown in the Pantanal, arthropods are more abundant in the warmer wet season (Santos Filho et al., 2008), and both host species may have higher consumption rates of these potential intermediate hosts during the wet season. However, while a primary food item consumed by both host species in the study area were coleopterans, which can be intermediate hosts for acanthocephalans, these were more frequently found in fecal samples of these animals in the dry season (Bianchi et al., 2014). The pre-patent period for acanthocephalans (infection of the intermediate hosts by cystacants and the development to adults) and the patent period can vary from weeks to months in acanthocephalans (Nicholas, 1967; Kennedy, 2006). If we consider the pre-patent period of acanthocephalans from mammals as 30 to 100 days (Nicholas, 1967; Crompton and Nickol, 1985), the acanthocephalan eggs would be more abundant in coati and fox feces in the wet season if those hosts were actually infected by mid-late dry season. However, the lack of knowledge regarding the life cycle and intermediate host species for these acanthocephalans precludes fully informed inferences regarding the mechanisms driving seasonal variation in parasite loads.

Overall, while the importance of seasonality for acanthocephalan was clear in both host species, the influence of host-related attributes varied for parasite-host interactions. Nonetheless, both host gender and host age

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appear to be important factors determining prevalence and parasite intensity of these acanthocephalans. The fact that general patterns of prevalence in the Pantanal did not differ between host species, and were similar for both genders in coatis and crab-eating foxes may indicate that differences in features such as body size and social behavior are relatively less important for predicting infection rates by acanthocephalans when compared to the availability and consumption rates of infected intermediate hosts by definitive hosts. Parasites loads, in turn, may shaped more by features related to host health and immune system function, which are in turn potentially affected by host age and gender.

Despite the study using survey approaches that focus on eggs rather than larval or adult stages, we were able to detect important patterns in acanthocephalan ecology, perhaps due to our relatively large sample sizes. We believe that using egg counts is a potentially powerful tool when sample sizes are large and when it is possible to obtain replicates from the same hosts. Morover, fecal egg counts represent a minimally invasive method for estimating parasite loads (Hämäläinen et al., 2015). The study of parasite dynamics in large animals using egg counts is particularly useful considering that many large host species show decreasing abundance and are already threatened by extinction (IUCN, 2008), which precludes host collection for parasite quantification.

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10.2 Chapter 2

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A new species of *Pachysentis* Meyer, 1931 (Acanthocephala: Oligacanthorhynchidae) in the brown-nosed coati *Nasua nasua* (Carnivora: Procyonidae) from Brazil, with notes on the genus and a key to species

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Running Title: A new species of *Pachysentis* from Brazil

Abstract

Pachysentis lauroi n. sp. (Oligacanthorhynchidae: Acanthocephala) is described from the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (Procyonidae: Carnivora) in the Brazilian Pantanal wetlands of the MatoGrosso do Sul State, Brazil. Specimens were studying using light and scanning electron microscopy. The new species is distinguished from other species of *Pachysentis* by the number of hooks in each longitudinal row (12 rows of 4 hooks, total of 48 hooks), presence of barbs on all hooks, and the organization of the cement glands. Notes on the genus *Pachysentis* Meyer, 1931 and a key to its species are provided. Critical comments on some species with a dubious diagnosis and questionable or missed key taxonomic characteristics are also reviewed. We also discuss the zoogeography of the members of the genus.

Keywords: Acanthocephala, *Pachysentis lauroi* n. sp., key to species, carnivore, MatoGrosso do Sul, Brazil.

Introduction

Pachysentis Meyer, 1931 comprises 10 species, which have been reported parasitizing mammals in Africa and the American continent (Meyer, 1931, Van Cleave, 1953, Golvan, 1957, Machado-Filho, 1950, García-Prieto et al. 2012; Vieira et al, 2008, Correa et al., 2016, Muniz-Pereira et al., 2016). Acanthocephalans

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of wild Brazilian mammals have been studied mainly by Travassos (1915, 1917, 1926, 1927) and Machado-Filho (1940, 1950), who described six species belonging to *Pachysentis*, five of these being reported in Brazil by Machado-Filho (1950) and Vieira et al. (2008). These species are (1) *Pachysentis gethi* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis gethi* Machado-Filho, 1950] from *Eira barbara* (Linnaeus,1758) (Carnivora, Mustelidae) in Pará and Rio de Janeiro States and from *Galictis cuja* (Molina, 1782) and *G. vittata* (Schreber, 1776) in Rio de Janeiro (Machado-Filho 1950; Vieira et al. 2008; Muniz-Pereira et al. 2016); (2) *Pachysentis procyonis* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis procyonis* Machado-Filho, 1950] from *Procyon cancrivorus* (Cuvier, 1798) (Carnivora, Procyonidae) in Rio de Janeiro State (Machado-Filho, 1950); (3) *Pachysentis rugosus* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis rugosus* Machado-Filho, 1950] from *Sapajus cay* (Illiger, 1815) (Primates, Cebidae) in Rio de Janeiro State; (4) *Pachysentis septemserialis* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis septemserialis* Machado-Filho,1950] from *Saguinus niger* (Hoffmannsegg, 1807) (Primates, Callitrichidae) in the Pará State (Machado-Filho, 1950; Correa et al., 2016); (5) *Pachysentis lenti* (Machado-Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis lenti* Machado-Filho, 1950] from *Callithrix geoffroyi* (Humboldt, 1812) (Primates, Callitrichidae) in Espírito Santo State.

The brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (Procyonidae) is a medium-sized carnivore abundant in many regions of South America (Alho et al. 1987; Bianchi et al. 2016), especially in the Pantanal wetlands region (Bianchi et al. 2014; Bianchi et al. 2016).A few species of acanthocephalans have been reported infecting *N. nasua*, including *Oncicola luehei* (Travassos, 1917) Schmidt, 1972 in Pará, São Paulo, Minas Gerais, Mato Grosso, and Mato Grosso do Sul States (Travassos 1917; Lent and Freitas1938; Machado-Filho 1950; Vieira et al. 2008) and *Neoncicola potosi* (Machado-Filho, 1950) Schmidt, 1972 in Foz de Iguaçú, Paraná State (Moraes 2016).

In this study, a new species, *Pachysentis lauroi* n. sp. is described using light microscopy and scanning electron microscopy (SEM) from the brown-nosed coati in the Brazilian Pantanal wetlands.

Material and Methods

Two adult brown-nosed coatis were found between 2007 and 2008 at the Nhumirin Ranch (18°59'S, 56°39'W), a research station of the Brazilian Agricultural Research Corporation (Embrapa/Pantanal) in the Nhecolândia subregion of the Pantanal, Mato Grosso do Sul State in the Brazilian Pantanal wetlands. The animals were collected during a research project investigating the ecology and health of wild carnivores. This research project included an inventory of helminth endoparasites. Acanthocephalan specimens were made

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available to parasitologists at the Oswaldo Cruz Foundation in Rio de Janeiro (FIOCRUZ/RJ). Animal procedures approved by the Brazilian Federal Environmental Agency (IBAMA, first license #183/2005, CGFAU/LIC; last license #11772-2) were followed.

The animals were necropsied and acanthocephalan specimens were collected from the small intestine of each individual host and stored in AFA (alcohol + formalin + acetic acid) for 24 hours and stored in 70% alcohol. Worms used for microscopical studies were stained with acid (hydrochloric) carmine, dehydrated in a graded ethanol series, cleared in phenol 90% and mounted in Canada balsam (modified from Amato 1985),examined using an Axion Scope A1Light Microscope (Zeiss,Göttingen, Germany), and illustrated with the aid of a drawing tube attached a Zeiss standard 20 light microscope (Zeiss, Göttingen, Germany).

Generic identification was based on the taxonomic key proposed by Schmidt (1972) and specific taxonomic descriptions. The description of the new species of *Pachysentis* was based on 11 specimens (six males and five females). Measurements are in millimeters unless otherwise stated. The range was followed by the mean in parentheses. Proboscis hooks were counted in longitudinal alternating rows; hooks were measured in terms of its total length: from basal region of hook to the tip, length of the root, and were measured hook + root (tip of the hook to base of the root). The accepted species of *Pachysentis* deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz - CHIOC (Helminthological Collection of the Oswaldo Cruz Institute), *P.gethi* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 15680, 17836 a, 17837 b-d, 17838 a-b, 17846, 17852, 38100), *P.rugosus* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 17827, 17828 b-c, 17848), *P.procyonis* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 17847, 17833 a-b, 17854), *P.septemserialis* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 10593, 17812 a-b), *P.lenti* (Machado-Filho, 1950) Schmidt, 1972 (CHIOC 14830, 17819 a, 17820 a-c) and species deposited in the Museum für Naturkunde, Berlin, *P.procubens* Meyer, 1931 (No. 2440, 2443, 2474, 6032), *P.ehrenbergi* Meyer,1931 (N°2426, 2432, 6033), *P.canicola* Meyer, 1931 (No.2571) were used for comparison. Specimens of *Pachysentis lauroi* n. sp were deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil, under the number CHIOC no. 38565a (holotype) and 38565b (allotype).

For SEM, the specimens were fixed for one hour at room temperature in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, washed in the same buffer and post-fixed for three hours at room temperature in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer. The material was then dehydrated in ascending ethanol series, critical point dried with CO_2, mounted with silver cello tape on aluminum stubs, and sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LV microscope (JEOL, Akishima,

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Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

Results

Description

Order Oligacanthorhynchida Petrochenko, 1956

Family Oligacanthorhynchidae Southwell et Macfie, 1925

Pachysentis lauroi n. sp. (Figs 1-11)

> **General:** With characters of *Pachysentis* as designated by Schmidt (1972). Trunk wider anteriorly. Proboscis subspherical with 12 longitudinal rows of four hooks each, totaling 48 hooks (Figs. 1 and 2). Proboscis hooks similar in size and shape in both sexes. Apical hooks (types I and II) large with posterior curvature, complex manubria and double roots expanding laterally (Fig. 2). Proximal rows with short hooks (types III and IV) and simple discoid roots (Fig. 2). Measurements of length of apical and proximal hooks: length of hook × length of root and [length from proximal extremity to distal extremity in parentheses] in micrometers: (I) 150-229 (182) × 142-203 (170) [197-207 (249)]; (II) 97-145 (115) × 58-113 (81) [126-184 (153)]; (III) 45-118 (70) × 21-53 (39) [61-129 (91)]; (IV) 26-87 (53) × 18-39 (27) [39-103 (63)]. Hooks with terminal barbs visible by light microscopy in all types of hooks (Figs. 2, 8, 9, 10). Base of proboscis surrounded by lateral papillae with elevated border and central pore (Figs. 1, 6, 7); single apical papilla present with elevated border and salient tip at center (Figs. 6, insert). No marked neck. Proboscis receptacle similar in shape and size in both sexes, with two sub regions measuring 0.87-1.33 (1.16) × 0.43-0.56 (0.47), with cephalic ganglion region (Fig. 1). Lemnisci long, flattened and curved (Fig. 5).

Males (based on six specimens): Trunk 6.00-16.61 (9.63) × 1.53-2.53 (1.91) wide anteriorly (Fig. 5). Proboscis 0.51-0.73 (0.64) × 0.68-0.85 (0.73) wide. Lemnisci 4.75-6.83 (5.60), reaching middle of trunk (Fig. 5). Reproductive system in posterior 2/3 of trunk. Testes almost equatorial, contiguous, ellipsoid, in tandem (Fig. 5). Anterior testis 0.85-1.76 (1.15) × 0.32-0.62 (0.48); posterior testis 0.90-1.90 (1.27) × 0.48-0.60 (0.55) (Fig. 5). Eight compact uninucleate cement glands, 0.72-1.22 (0.86) × 0.44-0.68 (0.56). Ejaculatory duct 1.10-2.13 (1.42). Copulatory bursa terminal, retracted in all specimens (Fig. 5).

> *Females* (based on five specimens): Trunk 10.79-12.95 (12.07) × 0.53-2.45 (1.62) anteriorly. Proboscis 0.53-0.87 (0.73) × 0.68-0.83 (0.78). Lemnisci 3.30 long in 1 specimen; others masked by eggs. Gonopore subterminal (Fig. 3). Vagina 0.16-0.21 (0.19) long (Figs. 3, 11); uterus 0.61-0.96 (0.80); uterine bell 0.23-0.38

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(0.31) × 0.29-0.32 (0.30) (n=2) (Fig. 3). Total reproductive system 1.11-1.34 (1.19) (n=3). Eggs ellipsoidal, with sculptured outer membrane, 0.064-0.082 (0.073) × 0.054-0.036 (0.045) (n=29) (Figs. 4).

Taxonomic Summary

Type host: *Nasua nasua* (Linnaeus, 1766) Storr, 1780 (brown-nosed coati).

Type locality: Nhumirim Ranch (18°85'90S, 56°83'90W), Mato Grosso do Sul State, Brazil.

Site of infection: Small intestine

Etymology: The new species is named in honour of Dr. Lauro Travassos, who contributed greatly to our knowledge of the Brazilian Acanthocephala.

Remarks

In this study, we identified the specimens obtained from *Nasua nasua* (Linnaeus, 1766) Storr, 1780 as belonging to the Oligacanthorhynchidae and *Pachysentis* due to the presence of a subspherical proboscis, anterior trunk wider than posterior, proboscis with 48 hooks in12 longitudinal rows of four hooks each using (Schmidt, 1972). In addition, Machado-Filho (1950) considered the number of hooks on the proboscis and the size of the testes as the best characteristics for identifying and distinguishing species of the genus. *Pachysentis lauroi* n. sp. is compared with the other valid species of *Pachysentis* in Table 1 and further distinguished in the dichotomous key presented below.

The status of *Pachysentis septemserialis* Machado-Filho, 1950

The specimens from CHIOC (17812 a-b and 10593) were carefully studied and it was observed that they exhibited some morphological characters not mentioned in the original description. The paratype (permanent slides CHIOC 17812 a-b) was not informative regarding the number of hooks, and a collar was observed at the base of the proboscis, suggesting affiliation with the genus *Prosthenorchis* Travassos, 1915. The female paratype from CHIOC 10593 has12 longitudinal rows of four hooks with total of 48 hooks, which contradicts the number of the hooks given in the original description (seven rows of seven hooks, total 49 hooks) with no collar at the base of the proboscis (Machado-Filho 1950). Additionally, there is a lack of some information on this species, such as the taxonomic and morphometric characters of adult males. Therefore, we suggest that the specimens designated as *P. septemserialis* (Machado-Filho, 1950) Schmidt, 1972 may be synonymous with *P. lenti* (Machado-Filho, 1950) Schmidt, 1972, as to the number of the hooks, other morphometric characteristics and the fact that both are parasites of primates of the family Callitrichidae. The taxonomy of this species needs to be revised.

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The status of *Pachysentis ehrenbergi* Meyer, 1931

      Specimens of *Pachysentis ehrenbergi* deposited in the Museum für Naturkunde from *Vulpes vulpes* (No. 2426) and *Naja haje* (No. 2432, 6033) were also examined. Specimens from both hosts had barbs on the tip of all hooks, which was not mentioned by Meyer (1931) in the original description. Other morphological characteristics, such as the number of hooks, short neck, the presence and size of nuclei in the leminisci and the reproductive organs agree with the original description.

      Pachysentis lauroi n. sp. distinguished from the other species of *Pachysentis* by a combination of morphological characters, including the number of the hooks in each longitudinal row, the presence of barbs on the hooks and the arrangement of the cement glands (Table 1). The following key and Table 1 do not include *P. septemserialis*, because of its uncertain taxonomic status, but enable the new taxon to be distinguished from the other nine recognized species of the genus.

1. Proboscis with 12 longitudinal rows, alternating or not, of 3 to 4 hooks —————————————————2

\- Proboscis with 12 alternating longitudinal rows of 7 to 9 hooks ——————————————— 9

2. Proboscis with a total of 42 to 48 hooks ——————————————————————— 3

\- Proboscis with a total of 72 hooks ————————————————*P. canicola* Meyer, 1931

3. Proboscis with a total of 42 hooks ———————————————————————————— 4

\- Proboscis with a total of 48 hooks ———————————————————————————— 5

4. Cement glands in pairs ———————————————————————————————————— 6

\- Cement glands clustered ———————————————————————————————————— 7

5. Hooks with visible barbs ("arrow-shaped hook tip") ——————————————————— 8

\- Hooks without barbs ——————————————————— *P. lenti* (Machado-Filho, 1950) Schmidt, 1972

6. Parasite of carnivores in Africa ———————————————*P. angolensis* (Golvan, 1957) Schmidt, 1972

\- Parasite of carnivores in the Americas ————————————— *P. gethi* (Machado-Filho, 1950) Schmidt, 1972

7. Very short lemnisci not reaching anterior testis. Parasites of carnivores ———————— *P. procyonis* (Machado-Filho, 1950) Schmidt, 1972

\- Leminisci reaching anterior testis. Parasites of primates ———————————— *P. rugosus* (Machado-Filho, 1950) Schmidt, 1972

8. Cement glands in pairs —————————————————————— *P. dollfusi* (Machado-Filho, 1950) Schmidt, 1972

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- Cement glands in clusters --- *P. lauroi* n. sp.

9. Proboscis 0.55 mm wide, with a total of 90 hooks without barbs ------------------- *P. procumbens* Meyer, 1931

- Proboscis 0.8-0.9 mm wide, with a total of 102 hooks with barbs --------------------- *P. ehrenbergi* Meyer, 1931

    Pachysentis lauroi n. sp. is further distinguished from *P. angolesis, P. canicola, P. procumbens, P. ehrenbergi, P. gethi, P. procyonis* and *P. rugosus* by the number of hooks in each row, with 12 longitudinal rows of four hooks each, totaling 48 hooks (Table 1). Our specimens were similar to *P. lenti* and *P. dollfusi* in the number of hooks (48) on the proboscis. The new species can, however, be distinguished from *P. lenti* by having barbs on all hooks and from *P. dollfusi* by the organization of the cement glands (in cluster *vs* in uniform pairs), the size of trunk and the definitive host (Table 1). In addition, when Machado-Filho (1950) described *P. dollfusi*, he indicated that this acanthocephalan infected a zoo animal in Brazil and that is native of Madagascar. Golvan (1994), however, warned that the origin of this species might not have been Madagascar. Nevertheless, it is not known whether the species originates in Brazil or Madagascar.

Discussion

    Meyer (1931) proposed *Pachysentis* with the type species *P.canicola* Meyer, 1931 from a domestic dog in Brazil. The same species was found infecting a gray fox *Urocyon cinereoargenteus* (Schreber, 1775) (Carnivora: Canidae) in the United States (Buechner 1944). Two additional species, *P. ehrenbergi* Meyer, 1931 and *P. procumbens* Meyer, 1931, were described from *Vulpes vulpes* (Linnaeus, 1758) in Egypt (Meyer 1931; Van Cleave 1953), suggesting that species from this genus are parasites of carnivores (Order Carnivora).

    Van Cleave (1953) also studied acanthocephalan parasites from North American mammals and recorded *P. canicola* in the gray fox and the skunks *Mephitis mephitis mesomelas* (Lichtenstein, 1832), *Conepatus leuconotus* (Lichtenstein, 1832) and *Spilogale gracilis leucoparia* (Merriam, 1890), and recognized the three previous species of the genus. Yamaguti (1963) revised the classification of the Acanthocephala and considered their geographic distributions, revised the diagnosis of the genus *Pachysentis* and followed the classification of Meyer (1931) and Van Cleave (1953) with three species in the genus.

    Schmidt (1972) revised the family Oligacanthorhynchidae and transferred six species of *Prosthenorchis* Travassos, 1915to the genus *Pachysentis*, i.e. *P. dollfusi, P. gethi, P. lenti, P. procyonis, P. rugosus, P. septemserialis* and *P. angolensis* [syn. *Oncicola angolensis* Golvan 1957]. *Pachysentis* Meyer, 1931 then included a total of 10 species based on morphological features, such as: an anterior trunk wider than the posterior trunk; the absence of a festooned collar; a globular proboscis with 12 longitudinal rows of 3 to 12 hooks, totaling 42 to 102 hooks; larger anterior hooks with complex manubria and roots, as well as rootless posterior hooks; tips

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of the hooks with or without barbs; long and flattened lemnisci in arranged a band; testes in tandem in the mid-trunk; eight compacted cement glands; and oval eggs with sculptured outer membranes (Yamaguti 1963; Schmidt 1972).

According to this classification, the type hosts for species of *Pachysentis* are primates and carnivores with geographic distributions restricted to Africa and North, Central and South America (Meyer, 1931, Van Cleave, 1953, Golvan, 1957, Machado-Filho, 1950, García-Prieto et al. 2012; Vieira et al, 2008, Correa et al., 2016, Muniz-Pereira et al., 2016). In the revisions by Golvan (1994) and Amin (2013), the authors updated the classification of the Acanthocephala and considered *Pachysentis* as including 10 valid species described by Meyer (1931), Golvan (1957) and Machado-Filho (1950). Therefore, the member species are *P. canicola, P. ehrenbergi, P. procumbens, P. angolensis, P. dollfusi, P. gethi, P. lenti, P. procyonis, P. rugosus* and *P. septemserialis*.

Our study provides details of *Pachysentis lauroi n. sp.* such as reproductive organs of females and males, as well as detail by scanning electron microscopy showing the presence of barbs on hooks in the proboscis, and the apical and lateral papillae-like structure on the proboscis. Furthermore, we are adding new information of morphology of two species, *P. septemserialis* and *Pachysentis ehrenbergi* and their status in the genus. These morphological features help to identify the new species and contributes to the taxonomy of this acanthocephalan genus. Finally, the present study also reports the definitive host – the brown-nosed coati *Nasua nasua* (Linnaeus, 1766) Storr, 1780 in a new geographical area, which enlarges the geographic distribution of the genus.

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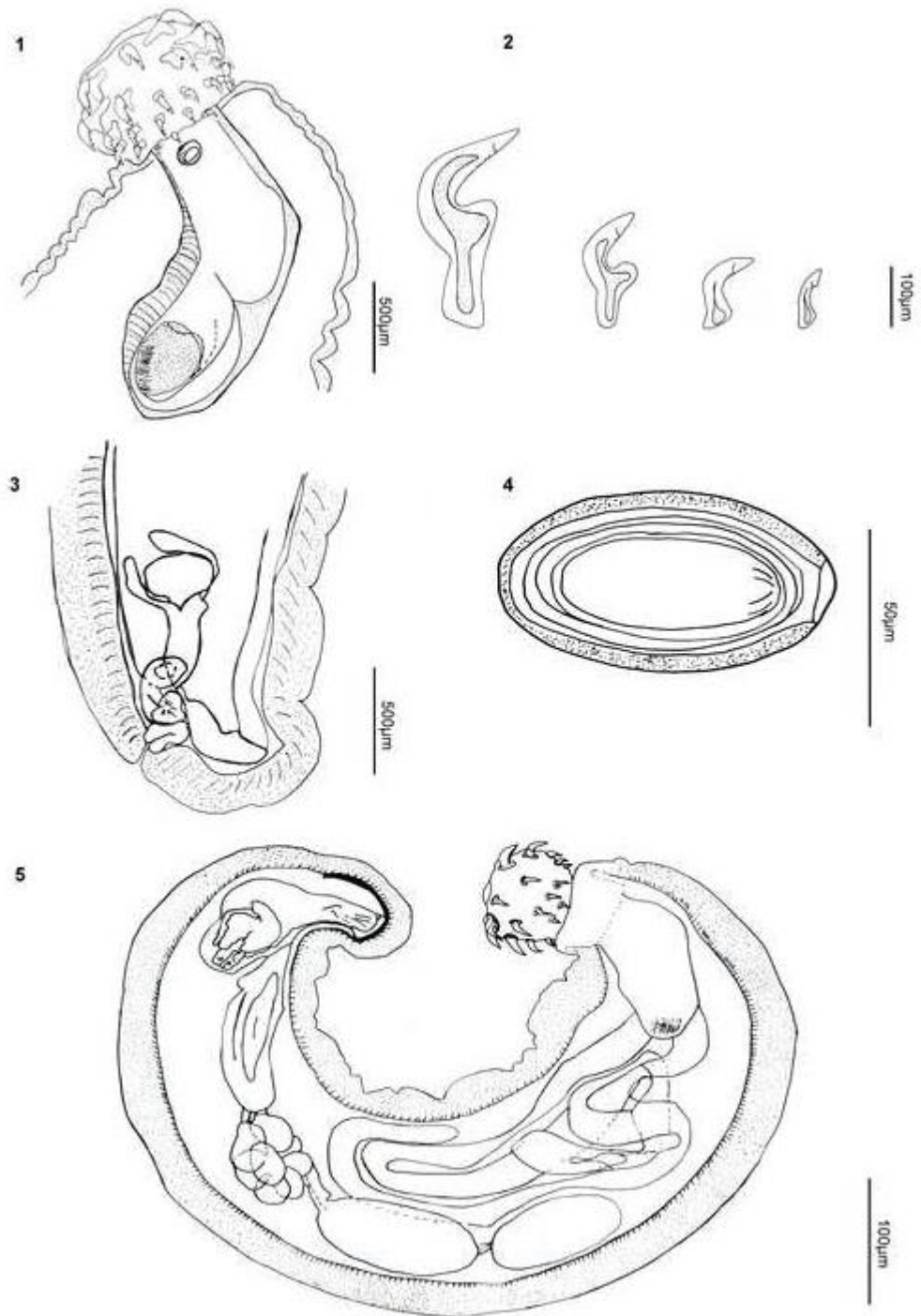
TABLES

Table I. Morphometric comparison of species of *Pachysentis* (measurements in mm)

FIGURES

Figs. 1-5 Line drawing of *Pachysentis lauroi* n. sp. collected in the intestine of *Nasua nasua* from the Brazilian Pantanal Wetlands, Mato Grosso do Sul State. 1. -globular proboscis with hooks and proboscis receptacle with cephalic ganglion in proximal region; 2. - row with 4 hooks, apical hooks with double root and proximal hooks with simple root; 3. - posterior region of female showing the vagina, uterus and uterine bell; 4. - ellipsoidal egg with 3 layers; 5. -adult male showing two testes, cements glands, ejaculatory ducts and retracted copulatory bursa.

Figs. 6-11 Scanning electron micrographs of specimens of *Pachysentis lenti* from *Nasua nasua* in the Brazilian Pantanal Wetlands, Mato Grosso do Sul State. 7 and 8. –globular proboscis with lateral papillae and apical papilla; 9 and 10. –apical and proximal hooks at base of the proboscis with barbs on the tips of the hooks (arrowhead); 11. -detail of the barbs on the tip of the apical hooks (arrowhead); 12. -posterior end of female body with subterminal vagina. Lpa, lateral papillae; Apa, apical papilla; Ne, neck; Pr, proboscis; Ho, hook; V, vagina
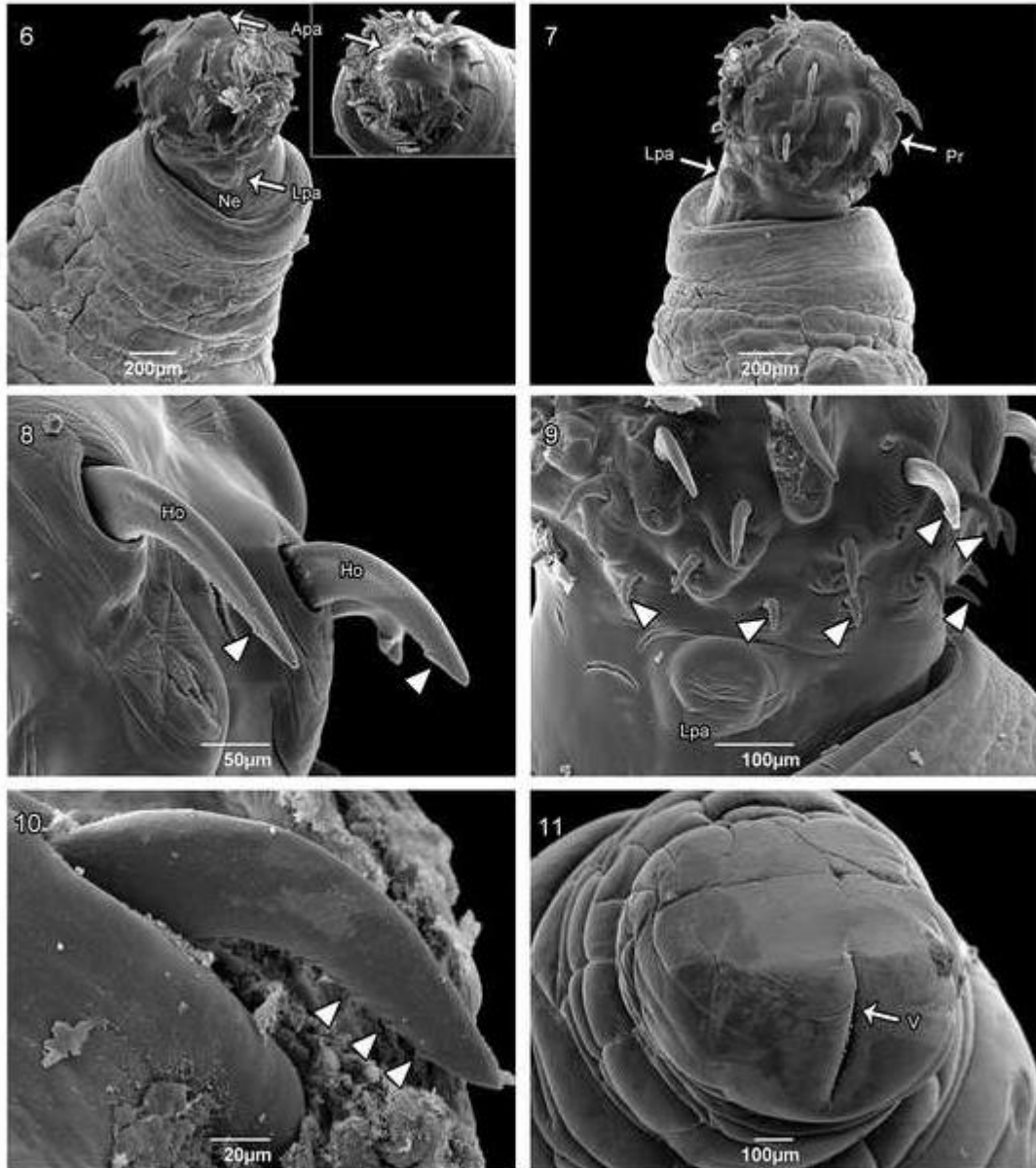
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Table 1. Morphometric comparison of species of the genus Pachysentis (measurements in mm)

Characteristics/Species	P. angolensis		P.canicola (type species)		P.procumbens (juvenile)		P.ehrenbergi		P.rugosus		P.procyonis	
Author	Golvan, 1957		Meyer, 1931		Meyer, 1931		Meyer, 1931		(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972	
type-host	*Canis adustus*		Dog (Meyer, 1931)		*Vulpes vulpes*		*Vulpes vulpes; Naja haje*		*Sapajus cay*		*Procyon cancrivorus*	
type-locality	Angola, Africa		Brazil, South America		Argo, Egito, Africa		Egito, Africa		Rio de janeiro, Brazil		Rio de janeiro, Brazil	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Trunk	17-23 X 3.5-4	34-48 X 4.8-5.5	15-28 X 4-8	20-26 X 5-11	6 X 1.25	6 X 1.25	25 X 4	26-29 X 6	25 X 3.5	32 X 3	20-30 X 2-3	25-35 X 2-3
Proboscis	0.55-0.63 X 0.70-0.82		0.57-0.80 X 0.57-0.85		0.55 X 0.55		0.8 X 0.9		0.564 X 0.694		0.697 X 0.716	
Total number of hooks	42		72		90		102		42		42	
Hooks per row	6 x 4 + 6 x 3		6 x 4 + 12 x 4*		6 x 7 + 6 x 8		6 x 9 + 6 x 8		6 x 4 + 6 x 3		6 x 4 + 6 x 3	
Barbs in hooks	no barbs		no barbs		no barbs		barbs		no barbs		no barbs	
Proboscis receptacle	1.5		2		1.2		1.3		1.24 X 0.481		1.37 X 0.531	
Lemnisci	5.8-6		7		-		7 X 0.8		4.64		3.64	
Anterior testis	2-3 X 0.9	-	2	-	-	-	3	-	1.57 X 0.697	-	3.01 X 1.24	-
Posterior testis	2-4.3 X 1.0	-	2	-	-	-	3	-	1.69 X 0.664	-	3.15 X 1.07	-
Dimension of group of cement gland	3	-	3	-	-	-	7	-	2.02	-	3.56	-
Ejaculatory duct length	2.3	-	-	-	-	-	-	-	1.68	-	3.53	-
uterine bell	-	-	-	3.15 - 8.15	-	-	-	-	-	5.86	-	4.64
eggs	-	0.09 X 0.043	-	0.07 x 0.045	-	-	-	0.07 X 0.05	-	-	-	0.071 X 0.042

Table 1. continued

Characteristics/Species	P.gethi		P.lenti		P.dollfusi		Pachysentis lourot n. sp. (present study)	
Author	(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972		(Machado Filho, 1950) Schmidt, 1972		present study	
type-host	Eira barbara		Callithrix geoffroyi		Eulemur fulvus (syn. Lemur fulvus)		Nasua nasua	
type-locality	Pará and Rio de Janeiro, Brazil		Espírito Santo, Brazil		Madagascar, Africa		Mato Grosso do Sul, Brazil	
	Male	Female	Male	Female	Male	Female	Male	Female
Trunk	10-15 X 1.0-2.5	15-25 X 1.5-3	15-20 X 1.0-2.5	20-25 X 2-2.5	50 X 4	50 x 4	9.63 X 1.91	12.07 X 1.62
Proboscis	0.583 X 0.794		0.63 X 0.664		-		0.68 X 0.76	
Total number of hooks	42		48		48		48	
Hooks per longitudinal row	6 x 4 + 6 x 3		6 x 4 + 6 x 4		6 x 4 + 6 x 4		6 x 4 + 6 x 4	
Barbs in hooks	no barbs		no barbs		barbs		barbs	
Proboscis receptacle	1.07 X 0.498		1.32		-		1.16 X 0.47	
Leminisci	3.48		3.15		4.3-6.6		4.45	
Anterior testis	1.40 X 0.581	-	1.76 X 0.51	-	-	-	1.15 X 0.48	-
Posterior testis	1.40 X 0.581	-	1.82 X 0.547	-	-	-	1.27 X 0.55	-
Dimension of group of cement gland	1.54	-	2.98	-	-	-	0.86 X 0.56	-
Ejaculatory duct length	4.64	-	-	-	-	-	1.42	-
uterine bell	-	5.56	-	1.41	-	-	-	1.19
eggs	-	0.084 X 0.054	-	-	-	0.08 X 0.05	-	0.073 X 0.045

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10.3 Chapter 3

Manuscript Details

Manuscript number	IJPPAW_2019_66
Title	New morphological and genetic Gigantorhynchus echinodiscus (Diesing, 1851) (Acanthocephala: Archiacanthocephala) in the giant anteater Myrmecophaga tridactyla Linnaeus, 1758 (Pilosa: Myrmecophagidae)
Article type	Full Length Article

Abstract

Gigantorhynchus echinodischus (Diesing, 1851) is a parasite of anteaters in South America. Although described by Diesing, 1851, there is still a lack of taxonomic and phylogenetic information regarding this species. In the present study, we redescribed G. echinodiscus collected from a giant anteater, Myrmecophaga tridactyla Linnaeus, 1758, from Brazilian Cerrado (Savannah) in the state of São Paulo by light and scanning electron microscopy. In addition, phylogenies were inferred from partial DNA gene sequence of the nuclear large subunit ribosomal RNA gene (28S rRNA). We provided details of the proboscis with a crown having 18 large hooks and numerous small hooks, a lateral papilla at the base of the proboscis, a ringed pseudo-segmented body, large testes, cemented glands in pairs, and a non-segmented region in the posterior end of the body, which contributed to the diagnostic of the species. Molecular phylogenetic analysis recovered G. echinodiscus forming a well-supported monophyletic group with Mediorhynchus sp., which was congruent with morphological studies that allocate both genera within the family Gigantorhynchidae. In conclusion, the present work added new morphological and molecular information, emphasizing the importance of adopting integrative taxonomic approaches in studies on Acanthocephala.

Keywords	Gigantorhynchidae; Integrative taxonomy; Phylogenetic systematics; 28S rRNA; Cerrado
Taxonomy	Parasitology, Helminthology
Manuscript region of origin	South America
Corresponding Author	A Maldonado
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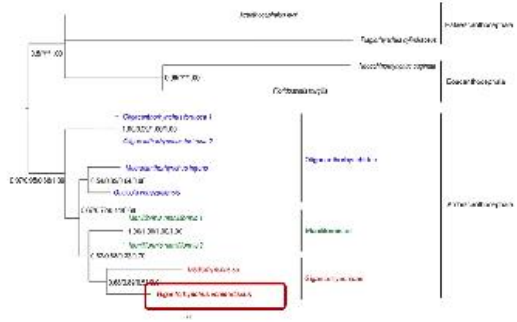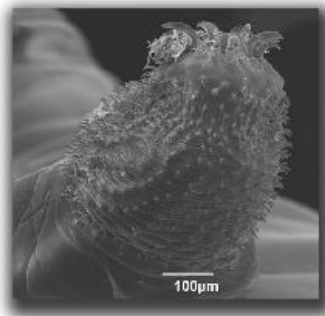Declaration of interest.docx [Conflict of Interest]

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

HIGHLIGHTS (3 – 5 points)

1. Redescription of *Gigantorhynchus echinodischus* from Brazilian giant anteater.
2. First molecular data of the genus *Gigantorhynchus* with 28S rRNA partial gene.
3. Phylogenetic relationships of Gigantorhynchidae are assessed.

Running head: GOMES ET AL.-.

New morphological and genetic *Gigantorhynchus echinodiscus* (Diesing, 1851)

(Acanthocephala: Archiacanthocephala) in the giant anteater *Myrmecophaga tridactyla*

Linnaeus, 1758 (Pilosa: Myrmecophagidae)

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ABSTRACT

Gigantorhynchus echinodischus (Diesing, 1851) is a parasite of anteaters in South America. Although described by Diesing, 1851, there is still a lack of taxonomic and phylogenetic information regarding this species. In the present study, we redescribed *G. echinodiscus* collected from a giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758, from Brazilian Cerrado (Savannah) in the state of São Paulo by light and scanning electron microscopy. In addition, phylogenies were inferred from partial DNA gene sequence of the nuclear large subunit ribosomal RNA gene (28S rRNA). We provided details of the proboscis with a crown having 18 large hooks and numerous small hooks, a lateral papilla at the base of the proboscis, a ringed pseudo-segmented body, large testes, cemented glands in pairs, and a non-segmented region in the posterior end of the body, which contributed to the diagnostic of the species. Molecular phylogenetic analysis recovered *G. echinodiscus* forming a well-supported monophyletic group with *Mediorhynchus* sp., which was congruent with morphological studies that allocate both genera within the family Gigantorhynchidae. In conclusion, the present work added new morphological and molecular information, emphasizing the importance of adopting integrative taxonomic approaches in studies on Acanthocephala.

Keywords: Gigantorhynchidae; Integrative taxonomy; Phylogenetic systematics; 28S rRNA; Cerrado

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1. Introduction

The family Gigantorhynchidae Hamman, 1892 is the unique family at the order Gigantorhynchida Southwell and Macfie, 1925 and contains two genera: *Mediorhynchus* Van Cleave, 1916 and *Gigantorhynchus* Hamman, 1892 (Amin, 2013). The genus *Gigantorhynchus* was validated by Yamaguti (1963) and Amin (1985, 2013), and comprises six valid species: *Gigantorhynchus echinodiscus* (Diesing, 1851) (type species) [syn. *Echinorhynchus echinodiscus* Diesing, 1851], *G. lopezneyrai* Diaz-Ungria, 1958, *G. lutzi* Machado Filho, 1941, *G. ortizi* Sarmiento1954, and *G. ungriai* Antonio, 1958 parasitizing marsupials and anteaters in South America (Yamaguti, 1963, Amin, 1985, 2013);and *G. pesteri* Tadros, 1966 parasitizing baboom in Africa (Tadros, 1966, Amin, 2013). Particularly, *G. echinosdiscus* is distributed over the Neotropical region and have been reported parasitizing anteaters in Brazil (Travassos, 1917, Machado Filho, 1941), Venezuela (Días-Ungria, 1958), Panamá (Dunn, 1934), and Trinidad Island (Camerón, 1939) (Table 1).

In Brazil, two species have been reported, *G. lutzi* Machado Filho, 1941 from the bare-tailed woolly opossum *Caluromys philander* Linnaeus, 1758 (Machado Fillho, 1941) and *G. echinodiscus* infecting anteaters, as the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758; the collaret anteater *Tamandua tetradactyla* (Linnaeus, 1758); and the silk anteater *Cyclopes didactylus* (Linnaeus, 1758) (Travassos, 1917, Strong et al., 1926, Machado Filho, 1941) (Table 1). Recently, eggs of *G. echinodiscus* have been recorded in coprolites of *T. tetradactyla* and *M. tridactyla* from an archaeological site in Brazil (Ferreira et al., 1989).

Currently records of *Gigantorhynchus* species are based on morphological data (Travassos, 1917, Machado Filho, 1941, Sarmiento, 1954, Antonio, 1958,Díaz-Ungría, 1958, Tadros, 1966) and genetic data is not available to the genus *Gigantorhynchus* in public databases.

Lately, the nuclear large subunit ribosomal gene (28S rRNA)have been used as molecular marker for phylogenetic inferences on acanthocephalans. For example, to elucidate the relationships

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amongst the four classes within the phylum Acanthocephala, to solve taxonomic problems at thefamilial level, and to investigateinter and intraspecific genetic variation within acanthocephalan species(García-Varela and Nadler, 2005, García-Varela et al. 2011, Braicovich et al., 2014, García-Varela and Pérez-Ponce de León, 2015, Pinacho-Pinacho et al., 2015, Wayland et al., 2015).Therefore, phylogenetic evidence based on 28S rRNA gene may be helpful, integrate complementing conventional taxonomic studies for different taxa.

In the present study,we redescribed *Gigantorhynchus echinodiscus* by light and scanning electron microscopy (SEM) and contributed with new molecular data and phylogenetic approach of the family Gigantorhynchidae.

2. Material and methods

2.1 Field study and recovery of acanthocephalan specimens

The giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 was subject of an ecological research program conducted by the São Paulo State University- UNESP/Jaboticabal (*Universidade Estadual Paulisa* - UNESP/Jaboticabal) and the Institute of Research and Conservation of Anteaters in Brazil (*Instituto de Pesquisa e Conservação de Tamanduás no Brasil - Projeto Tamanduá*), aiming to monitor movement and space use by giant anteaters using GPS devices. The study was conducted in Santa Bárbara Ecological Station (*Estação Ecológica de Santa Bárbara* – ECc Santa Bárbara, 22°48□59″S,49°14□12″W) located in the municipality of Águas de Santa Bárbara, state of SãoPaulo, Southeastern Brazil. The ECc Santa Bárbara encompases 2,712 ha of isolated and protected Cerrado remnant in the state of São Paulo and is characterized by a mosaic vegetation of Cerrado *sensu lato*, gallery forest, patches of semideciduous forest, and plantation of exotic *Pinus* and *Eucalyptus* species (Mello and Durigan, 2011).

Anteaters were captured and sedated for biometric measurements, sample collection, and GPS placement (Bertassoni et al, 2017).Two giant anteaters necropsied revealed presence of parasites in the intestine. After necropsy, the digestive tract was analyzed and helminths were collected from the small intestine, stored in 70% ethanol, and donated to the Laboratory of Biology

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and Parasitology of Wild Reservoir Mammals (*Laboratório de Biologia e Parasitologia de Mamíferos Silvetres Reservatórios* - LABPMR). At the LABPRM, the acanthocephalan specimens used for morphological characterization were stained with acid carmine, destained in a solution of 2% hydrochloric acid (HCl) and 70% ethanol, dehydrated in a graded alcohol series (70 to 100%), clarified in 90% phenol, whole-mounted as definitive slide in Canada balsam (modified from Amato, 1985), and analyzed using an Axion Scope A1 Light Microscope (Zeiss, Göttingen, Germany). Drawings were made with the aid of camera lucida attached to a Nikonlight microscope Model Eclipse E200MVR (Nikon Corporation, Tokyo, Japan). Measurements were in millimeters unless otherwise stated, range followed by mean within parentheses. The length of proboscis was the measurement of the neck, with small hooks, plus the crown of hooks (praesoma). We made three length measurements of the hooks with double root: from the tip of the hook to the root, total length of the hook; and total length of the root. Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (*Coleção Helmintológica do Instituto Oswaldo Cruz* - CHIOC), Rio de Janeiro, Brazil under the number CHIOC n° 38580.

For scanning electron microscopy (SEM) the specimens previously fixed in 70% ethanol were dehydrated in ascending ethanol series (80%, 90%, 100%), dried by the critical point method with CO_2, mounted with silver cellotape on aluminum stubs, and sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LVmicroscope (JEOL, Akishima, Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute (Plataforma de Microscopia Eletrônica Rudolf Barth/IOC- FIOCRUZ).

2.2 Molecular analyses

For gene sequence studies, specimens preserved in 70 % ethanol were washed in ultrapure water for 24 hours at room temperature. Total genomic DNA was isolated using the QIAamp DNA mini Kit according to the manufacturer's protocol (Qiagen, Venlo, The Netherlands). DNA amplifications by polymerase chain reaction (PCR) were conducted for the partial nuclear large subunit ribosomal RNA gene (28S rRNA) using the primersC1 5′-ACCCGCTGAATTTAAGCAT-

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3′ and D2 5′-TGGTCCGTGTTTCAAGAC-3′ (Chisholm et al., 2001). PCR amplifications were performed using Promega PCR Master Mix (Promega Corporation, Madison, Wisconsin, USA). Reactions were 25 μL following the manufacturer's protocol. The thermal-cycling profile was programmed on a thermocycler Eppendorf Mastercycler Epsystem (Eppendorf, Hamburg, Germany) with an initial denaturation step of 95 °C/ 2 min; followed by 40 cycles of 94 °C/ 60 s, 55 °C/ 60 s, and 72 °C/ 60 s; a final extension at 72 °C/ 5 min; and a cool down to 4°C. PCR products were analyzed after electrophoresis on 1.5% agarose gel using GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) by visualizing on UV transilluminator. Successful amplifications were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Sequencing reactions using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) were performed using the same primers mentioned above in a Gene Amp (Applied Biosystems) thermocycler and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems). Both procedures and cycle-sequenced products precipitationswere conducted at thesubunit RPT01A – DNA sequencing platform of the Oswaldo Cruz Institute PDTIS/FIOCRUZ.

Chromatograms were initially assembled into contigs, and manually edited for ambiguities using the software package Geneious 9.1.8 (http://www.geneious.com; Kearse et al., 2012). For assessment of phylogenetic relationships of *G. echinodiscus* sequence, we built a matrix with sequences of representatives of the class Archiacanthocephala retrieved from GenBank. Three families, representing three different orders of archiacanthocephalans, were present in our dataset: Oligacanthorhynchidae represented by sequences of the genera *Oligacanthorhynchus*, *Macracanthorhynchus*, and *Oncicola*; Moniliformidae represented by sequences of the genus *Moniliformis*; and Gigantorhynchidae represented by a sequence of the genus *Mediorhynchus* and our sequence of *Gigantorhynchus*. All of these genera infect mammals and *Mediorhynchus* may infect birds, as well. As outgroup we used two genera of the class Palaeacanthocephala

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(*Acanthocephalus* and *Plagiorhynchus*) and two genera of the class Eoacanthocephala

(*Neoechinorhynchus* and *Floridosentis*) (Table 2).

We aligned all sequences using the Program MAFFT under default parameters in the Geneious

package, followed by manual edition of the sequences, removing the non-complementary regions.

The sequences were realigned using the Geneious alignment algorithm using as settings global

alignment with free end gaps, cost matrix of transition/transversion (5.0/1.0), and same penalty

value of six for both gap opening and extension. The resulting aligned matrix was manually

trimmed of poorly aligned regions using the Mesquite 3.51 software package (Maddison and

Maddison, 2018).

As assessment of the quality of the data, we tested for the presence of phylogenetic signal

the Permutation Test Probability - PTP and the G1 tests in the program PAUP 4.0a164 (Swofford,

2003); and for the presence of substitution saturation using the Xia test (Xia et al., 2003, Xian and

Lemey, 2009) with analysis performed on fully resolved sites only and a graphic of transitions and

transversions versus JC69 model genetic distances (Jukes and Cantor, 1969) in DAMBE 7.0.35

(Xia, X., 2017).

Phylogenetic relationships based on partial 28S rRNA gene sequences were inferred using

Maximum Parsimony (MP), maximum-likelihood (ML), and Bayesian Inference (BI) methods. MP

was carried out using PAUP 4.0a164 (Swofford, 2003) with tree heuristic search using starting trees

via stepwise addition, with 100 random sequence addition replicates, holding 10 trees at each step,

and tree bisection and reconnection (TBR) branch-swapping algorithm. Node supports in MP were

assessed by non-parametric bootstrap percentages (MP-BP) after 10,000 pseudoreplications.ML

was carried out using PhyML 3.0 (Guidon et al., 2010) with tree heuristic search using subtree

pruning and regrafting (SPR), with 10 random starting trees, with model selection by the SMS

algorithm (Smart Model Selection) (Lefort et al., 2017) under the Akaike information criterion

(AIC). Node supports in ML were assessed by approximate likelihood-ratio test (aLRT) for

branches (Anisimova and Gascuel, 2006) and by non-parametric bootstrap percentages (ML-BP)

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after 1,000 pseudo-replications. BI was carried out using MrBayes version 3.2.6 (Ronquist et al., 2012) on the CIPRES Science Gateway platform V. 3. 3 (Miller et al., 2010) with tree heuristic search using SPR, with 10 random starting trees, with model selection by the SMS algorithm under the Bayesian information criterion (BIC), with two simulation runs of the Markov chain Monte Carlo (MCMC), for 10 million generations, sampling every 100 generations, and with a 'burn-in' removal of 25%. Node supports were assessed in BI by Bayesian posterior probabilities (BPP). Effective Sample Sizes (ESS) of parameters were estimated using Tracer v1.7.1 (Rambaut et al., 2018) to assess sampling robustness. We considered values over 100 effectively independent samples sufficient.

3. Results (*Figs. 1-16*)

3.1 Redescription

Family Gigantorhynchidae Hamann, 1892

Genus *Gigantorhynchus* Hamann, 1892

Gigantorhynchus echinodiscus (Diesing, 1851)

General. Gigantorhynchida: Gigantorhynchidae. With characters of the genus *Gigantorhynchus*.

Body of median size, narrow, and apparently segmented. Sexual dimorphism in body size, with females larger than males. Proboscis cylindrical (Figures 1, 6 and 12) and similar in both sexes with a single crown of large hooks in the apex of the proboscis (Figures 6 and 8), formed by two rows of hooks in a total 18 hooks with double roots (Figures 1, 8 and 12). The first row with six robust hooks and the second row with 12 hooks in pairs, smaller than those in the first row (Figure 2 and 8). Measurement of the hooks with double root: from the tip of the hook to the root, total length of the hook; and total length of the root: six hooks of the first row measured 0.16-0.23 (0.20); 0.12-0.18 (0.15); 0.11-0.16 (0.14). The 12 hooks of the second row measured 0.18-0.19 (0.18); 0.11-0.13 (0.12); 0.11-0.12 (0.11).The crown is separated from numerous small-rootless hooks by a slight space without hooks (Figure 6). The small-rootless hooks were arranged in longitudinal rows

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(Figure 1, 2, 6 and 7) and measured 0.05-0.08 (0.07). Two lateral papillae in the neck were observed with a slightly elevated border (Figure 1, 7 and 9). Behind the proboscis, it was observed a region without segmentation. The lemnisci were long and filiform in both sexes.

Male (nine specimens): Body 45.29-14.80 (31.53) long and 0.99-0.53 (0.78) wide. Proboscis and neck 0.65-0.45 (0.55) long and 0.30-0.55 (0.45) wide having a crown with 18 hooks followed by numerous and small-rootless hooks arranged on longitudinal rows. After the proboscis a region without segmentation measuring 2.24-3.21 (2.72) long. The proboscis receptacle 0.48-0.64 (0.57) long and 0.21-0.32 (0.26) wide. The lemnisci 8.02-20.30 (14.87) (n=3), reaching the anterior testis. The testes were ellipsoids, narrow, and in tandem; the anterior testis 1.63-2.71(2.25) long and 0.26-0.32 (0.29) wide; posterior testis 1.61-2.66 (2.13) long, and 0.26-0.39 (0.29) wide (Figure 3). Eight cement glands in pairs, the group measuring 0.98-2.13(1.61) long and0.45-0.76 (0.60) wide (Figures 3 and 14) followed by an ejaculatory duct 0.82-1.42 (0.97) long. The posterior end after the anterior testes did not have a segmentation region and measuring 5.45-8.53 (6.83) and had smooth surface with a copulatory bursa at the end (Figure 3 and 14).The gonopore terminal had invaginated bursa.

Female (six specimens): Body 102.79-52.92 (75.45) long and 0.79-1.13(0.85) wide. Proboscis and neck 0.49-0.71 (0.55) long and 0.46-0.53 (0.48) wide. Proboscis receptacle 0.63-0.74 (0.70) long and 0.23-0.31 (0.27) wide. The lemnisci were long and difficult to see due to the covered by eggs in most specimens and measured 13.23 mm long (n=1). The vagina was subterminal and had a "guitar" form (Figures 4, 15, and 16). The uterine bell to genital pore including the vagina, uterus, and uterine bell measured 0.69-0.97(0.86) (n=5) (Figure 4). Eggs were ellipsoids with three membranes 0.059-0.069 (0.064) long and 0.04-0.03(0.036) wide (n=26; Figures 5 and 13).

Taxonomic summary

Host: *Myrmecophaga tridactyla* Linnaeus, 1758

Site: Small intestine.

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Locality: Santa Bárbara Ecological Station – ECc Santa Bárbara (22°48□59″S, 49°14□12″W), São Paulo, Brazil.

Specimens deposited: CHIOC n°. 38580

3.2 Molecular Analyses

Sequencing result in a partial 28S rRNA gene consensus sequence of 771bp from one adult *G.igantorhynchus echinosdiscus*. The resulting matrix was comprised of 12 taxa and 534 characters, of which 68 characters were constant (proportion = 0.1273), 194 were parsimony-uninformative and 272 were parsimony-informative variable characters. The PTP (P = 0.0001) and the G1 (G1 = 0.9227) tests indicated the presence phylogenetic signal and the test by Xia provided no evidence for substitution saturation in the 28S rRNA data matrix.

The MP analysis resulted in a 1053 steps length single most-parsimonious tree with 0.7179 consistency index (CI), 0.2821 homoplasy index (HI), and 0.3695 rescaled consistency index (RC).The ML best-fit model chosen by SMS on PhyML under AIC was the TN93+G, with 4 substitution rate categories, and gamma shape parameter 1.217, resulting in a tree with score lnL= -3556.2275.The best-fit model used to infer BI under BIC chosen by SMS on PhyML was HKY+G and the BI resulted in a mean estimated marginal likelihood -3571.9031 (median = 3571.5520, standard deviation = 39.3280). Estimated sample sizes (ESS) were robust for all parameters.

Our phylogenies inferred using MP, ML and BI resulted in similar topologies with variations in nodes and support values. The BI topology is shown in Figure 17. The class Archiacanthocephala was monoplyletic with strong support (MP-BP = 0.97, aLRT = 0.95, ML-BP = 0.88, BPP = 1.00). All analyses agreed that the sequence of *G. echinodiscus* formed a moderately to well-supported monophyletic group with *Mediorhynchus* sp. (MP-BP = 0.68, aLRT = 0.91, ML-BP = 0.55,BPP = 0.91).The family Gigantorhynchidae (*Gigantorhynchus* and *Mediorhynchus*) was sister to the family Moniliformidae (MP-BP = 0.67, aLRT = 0.68, ML-BP = 0.32,BPP = 0.70) represented by sequences of *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 that formed a well-supported monophyletic group (MP-BP = 1.00, aLRT = 1.00, ML-BP = 1.00, BPP =

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1.00). The group formed by Gigantorhynchidae and Moniliformidae was sister to a grouped formed by sequences of *Macracanthorhynchus ingens* (von Linstow, 1879) Meyer, 1932 and *Oncicola venezuelensis* Marteau, 1977 (MP-BP = 0.54, aLRT = 0.72, ML-BP = 0.42, BPP = 0.68), although with low support. In addition, the sequences of *Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972 formed a well-supported monophyletic group (MP-BP = 1.00, aLRT = 0.99, ML-BP = 1.00, BPP = 1.00) sister to all the other archiacanthocephalans.

3.3 Remarks

Species of the genus *Gigantorhynchus* are characterized by the presence of a cylindrical proboscis with a crown of robust hooks followed by numerous small hooks, long body with segmentation, long and filiforms lemnisci, and ellipsoid testes (Travassos, 1917; Sothwell and Macfie, 1925, Yamaguti, 1963), and parasites marsupials and anteaters in South America and one infecting a baboon in Africa (Table 3).

The specimens we found parasitizing *M. tridactyla*, were identified as *G. echinosdiscus* due to the presence of a single crown with two rows of 6 and 12 hooks, totalling 18 hooks, ringed pseudo-segmented body, long testes, and eight cement glands in pairs. This species is distinguished from *G. lutzi*, *G. lopezneyrai*, *G. ortizi*, and *G. pesteri* by the number and size of hooks of the crown in the proboscis, type of pseudosegmentation, and size of the eggs (Table 3).

The number and the size of hooks on the proboscis of *G. echinosdischus* in the present study was similar to that of *G. echinosdiscus* and *G. ungriai* described by Travassos (1917) and Diás-Ungría (1958), respectively. However, the type of segmentation was distinguished from *G. ungriai*, which has ringed complete segmentation with union in dorsal and ventral regions whereas *G. echinosdicus* lacks ringed form with incomplete segmentation (Table 3).

4. Discussion

The genus *Gigantorhynchus* was erected by Hamman, 1892 as the single genus of the family Gigantorhynchidae with the type species *Gigantorhynchus echinodiscus* (syn. *Echinorhynchus echinosdiscus*) (Diesing, 1851). In 1917, Travassos revised the family Gigantorhynchidae and

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separated the family in two subfamilies: Gigantorhynchinae and Prosthenorchinae. The genus *Gigantorhynchus* was included in the subfamily Gigantorhynchinae with four more genera: *Moniliformis* (Travassos, 1915), *Oligacanthorhynchus* (Travassos, 1915), *Empodius* (Travassos, 1916), and *Hamanniella* (Travassos, 1915), parasites of mammals and birds. Van Cleave (1923) reviewed Acanthocephala proposing a classification key to the genera considered valid, including the genus *Gigantorhynchus* that includes parasites of mammals from the Neotropical region. Later, Southwell and Macfie (1925) divided Acanthocephala in three sub-orders: Neoechinorhynchidea, Echinorhynchidea and Giganthorhynchidea the last having only the genus *Gigantorhynchus* with one species *Gigantorhynchus echinodiscus*. Meyer (1931), studying acanthocephalans from the Berliner Museum considered valid two more genera *Mediorhynchus* (Van Cleave, 1916) and *Empodius* (Travasso, 1915). However, Ward (1952) reviewed the acanthocephalans and moved *Heteracantorhynchus* Lundström, 1942 and excluded *Empodius* from the family Gigantorhynchidae. Thereafter, Van Cleave (1953) reporting acanthocephalans from North American mammals, considered the genus *Empodius* synonymous to the genus *Mediorhynchus* and established only two genera within the family Gigantorhynchidae: *Gigantorhynchius* and *Mediorhynchus*. Next, Yamaguti (1963) revised the classification of the family Gigantorhynchidae and reconsidered four genera within the family: *Gigantorhynchus, Empodius, Mediorhynchus, and Heteracanthorhynchus*, with *Gigantorhynchus* including five valid species. Golvan (1994) revised the nomenclature of the phylum Acanthocephala considering the geographical distribution as a taxonomic criterion and included more 24 species to the genus *Gigantorhynchus* as synonyms of different genera. Indeed, Amin (2013) recently updated the classification of family Gigantorhynchidae including two genera: *Gigantorhynchus* and *Mediorhynchus,* in agreement with Van Cleave (1953). In addition, he considered valid six species: *G. echinosdichus* (Diesing, 1851), *G. lutzi* Machado Filho (1941), *G. ortizi* Sarmiento (1953), *G. ungriai* Antonio (1958),*G. lopezneyrai* Díaz-Ungría (1958) and *G. pesteri* Tadros(1966), parasites of mammals (anteaters, didelphid marsupials, and a baboon) from South America and South Africa.

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Amato et al. (2014) reported, for the first time in Brazil, cystacanths of *G. echinosdiscus* infecting termites as intermediate hosts. Termites are nearly the entire portion of the giant anteater's diet (Rodrigues et al., 2008, Gaudin et al., 2018), suggesting that these arthropods are intermediate hosts of *G. echinosdiscus*.

Our molecular phylogenetic analyses, suggested that *G. echinosdiscus* is closely related to *Mediorhynchus* sp. by forming a well-supported monophyletic group, and being consistent with morphological data that group these two genera within the family Gigantorhynchidae.

Furthermore, our phylogenetic analyses of the class Archiacanthocephala genera agreed with previous studies recovering the family Gigantorhynchidae as sister to Moniliformidae, although with moderate support values. Additionally, according to previous studies with other molecular markers, such as CO1 and 18S, without *Gigantorhynchus*, the genus *Mediorhynchus* is sister to genus *Moniliformis* (García-Varela and Nadler, 2005, Amin et al., 2013, García-Varela and Pérez-Ponce de León, 2015, Amin et al., 2016). Noteworthy, was the basal, non-monoplyletic Oligacanthorhynchidae, suggesting that relationships may not be well resolved within this group, and the characters differing this group may be plesiomorphic, requiring further thorough studies.

In conclusion, our 28S rRNA gene study provided the first DNA sequence and the first phylogenetic analyses for the genus *Gigantorhynchus*.Thus, extending knowledge about acanthocephalans from Brazilian mammals and emphasizing the importance of integrative taxonomic studies to clarify their taxonomy.

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Legends to Figures

Figs. 1-5 Line drawing *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 1. Praesoma with the proboscis presenting a crown with robust hooks followed by small hooks; 2. Three different robust hooks in the crown and a small one type in the proboscis; 3. Posterior region of adult male showing reproductive organs; 4. Posterior region of adult female showing the uterus, vagina and gonopore subterminal; 5. Egg.

Figs. 6-11. Scanning electron micrographs of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 6 and 7. Cylindrical proboscis armed with hooks (Ho) showing a space (Sp) between the two circles of large hooks and small rootless hooks, neck (Ne), trunk (Tr), lateral papillae (Pa); 8. Detail of the crown with two circles of large hooks; 9. Detail of the lateral papillae; 10 and 11. Posterior end of adult male showing the region without pseudo-segmentation (cross) and a copulatory bursa protruded body (Cb).

Figs. 12-16 Light microscopy of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 12. Proboscis with a crown of large hooks in the apex and small hooks; 13. Egg; 14. Testis, cement glands in pair, ejaculatory duct; 15 and 16. Detail of the posterior end of adult female showing the uterus, vagina and gonopore subterminal.

Fig. 17. Bayesian Inference phylogenetic reconstruction tree of 28S rRNA gene sequences of *G. echinodicus* in the present study (in bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups. Nodes values are MP-BP, aLRT, ML-BP, and BPP, respectively.* no support or node not recovered in the respective analysis.

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Table 1. Reports and geographic distribution of *Gigantorhynchus echinodiscus* in mammals of South America.

Species of host	Family of host	Locality	Author
Cyclopes didactylus	Cyclopedidae		
		Brazil	Travassos, 1917
Myrmecophaga tridactyla		São Paulo, Brazil	Travassos, 1917
		Brazil	Diesing, 1851; Haman, 1892
		Rio de Janeiro and São Paulo, Brazil	Travassos, 1917
		Amazon, Brazil	Strong et al., 1926
	Myrmecophagidae	Panama City, Panama	Dunn, 1934
Tamandua tetradactyla		Trinidad Island	Cameron, 1939
		Pará, Brazil	Machado Filho, 1941
		Atures, Venezuela	Díaz-Ungria, 1958
		Brazil	Diesing, 1851; Haman, 1892

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Table 2. Accession numbers of sequences from GenBank used in our phylogenetic analyze using with 28S rRNA gene.

Class	Family	Species	Acession number	Reference
		Oligacanthorhynchus tortuosa 1	AY210466	Passamaneck and Halanych (2006)
		Oligacanthorhynchus tortuosa 2	KM659327	Lopez-Caballero et al. (2015)
		Macracanthorhynchus ingens	AY829088	Garcia-Varela and Nadler (2005)
Archiacanthocephala	Oligacanthorhynchidae	*Oncicola venezuelensis*	KU521567	Santos et al. (2016)
		Moniliformis moniliformis 1	AY829086	Garcia-Varela and Nadler (2005)
		Moniliformis moniliformis 2	MF398414	Mendenhall et al. (2018)
		Mediorhynchus sp.	AY829087	Garcia-Varela and Nadler (2005)
		Gigantorhynchus echinodiscus	**MK635344**	**present study**
Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus lucii*	AY829101	
	Plagiorhynchidae	*Plagiorhynchus cylindraceus*	AY829102	Garcia-Varela and Nadler (2005)
Eoacanthocephala	Neoechinorhynchidae	*Neoechinorhynchus saginata*	AY829091	
		Floridosentis mugilis	AY829111	

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Table 3. Morphometric comparisons of *Gigantorhynchus* species (measurements in milimmiters).

Species	*Gigantorhynchus echinodiscus*		*Gigantorhynchus echinodiscus*		*Gigantorhynchus lutzi*		*Gigantorhynchus lopezneyrai*	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Trunk Length	50-75	150-220	18.0	-	35-60	130-200	16-58	
Trunk Width	1-2.0	1.5-3.0	1.0	-	0.75-1.15	1-2.5	1-1.7	-
Anterior end without segmentation	4.0-5.0		3.0		-		no region without segmentation	
Proboscis+neck Length	1.0		1.0		1.695		1.131-1.5	
Proboscis+neck Width	0.5		0.3		0.735		0.66	
Number of hooks	18 (6+12)		18 (6+12)		12 (6+6)		12 (4+8)	
Hook to root x root	0.20 x 0.13 (1st row), 0.15 x 0.08 (2nd row)		0.18 (1st row) x 0.14 (2nd row)		0.285 x 0.165 (1st row), 0.225 x 0.135 (2nd row)		0.235 (1st row), 0.106 (2nd row)	
Small hooks length	0.04		0.04		0.048		-	
Receptacle	-		-		-		-	
Lemnisci	20-30		7.9-9.0		2.595		8	
Anterior testis Posterior testis	6-8.0 x 0.5-0.8		1.0 x 0.4		5.752-6.045 x 0.750-0.900		0.7 x 0.190	
Number of cement glands	8		8		8		8	
Dimension group of cement glands	4-5.0		-		-		-	
Organization of cement glands	in pairs		in pairs		in pairs		in pairs	
Ejaculatory duct	1.5-2.0		-		2.10-2.55		-	
uterine bell	-		-		1.575 x 0.270		-	
eggs	0.064 x 0.042		0.064-0.07 x 0.042-0.045		0.115 x 0.064		-	
Type of body segmentation	ringed form and no complete		ringed form and no complete segmentation		ringed form and no complete segmentation		slightly segmented	
Author	Travassos, 1917		Díaz-Ungría, 1958		Machado Filho, 1941		Díaz-Ungría, 1958	
Geographic distribuition	Rio de Janeiro, São Paulo, Brazil; Trinidad island; Panama; Venzuela		Atures, Venezuela		Pará, Brazil; Huanuco, Peru		Venezuela	
Vertebrate Host	*Tamandua tetradactyla, Cyclopes didactylus, Myrmecophaga tridactyla*		*Tamandua tetradactyla*		*Caluromys philander; Didelphis marsupialis*		*Tamandua tetradactyla*	
Reference	Travassos, 1917; Strong et al., 1926; Dunn, 1934; Cameron, 1939; Antonio, 1958		Díaz-Ungría, 1958		Machado Filho, 1941; Tantalean et al., 2005		Díaz-Ungría, 1958	

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Table 3. continued

Species	Gigantorhynchus ortizi		Gigantorhynchus pesteri		Gigantorhynchus ungriai		Giganthorhynchus echinodiscus (present study)	
Sex	Male	Female	Male	Female (immature)	Male	Female	Male	Female
Trunk Length	46-75	130-242	-	15-18	22-36	129-136	31.53	75.45
Trunk Width	1.4-1.92	1.5-2.0	-	0.8-0.9	0.78-1.58	1-1.6	0.78	0.85
Anterior end without segmentation					2-2.6		2.72	
Proboscis+neck Length	1.45-1.72			0.35	0.189-1.0		0.50	0.55
Proboscis+neck Width	0.435-0.555			0.1	0.237-0.7		0.30-0.52 (0.42)	0.48
Number of hooks	12 (6+6)			4	18 (6+12)		18 (6+12)	
Hook to root x root	0.160 x 0.10 (1st row), 0.140 x 0.09 (2nd row)			0.03	0.140-0.2 (1st row), 0.104-0.180 (2nd row)		0.20 (1st row) x 0.14 (1st row), 0.18 (2nd row) x 0.11 (2nd row)	
Small hooks length	0.05			0.015	0.02-0.06		0.07	
Receptacle	0.750-0.920			0.75 x 0.18-0.2	-		0.57 x 0.26	0.70 x 0.27
Lemnisci	5.48-6.80			3.6-4	1.75-3.27		14.87	
Anterior testis	1.98-3.0 x 0.56-0.96		-		2.0-5.6 x 0.395-0.474		2.25 x 0.29	
Posterior testis							2.13 x 0.29	
Number of cement glands	8		-		8		8	
Dimension group of cement glands	-		-		0.869 x 0.1896		1.61 x 0.60	
Organization of cement glands	in group		-		-		in pairs	
Ejaculatory duct		-	-		2.6		0.97	
uterine bell		-		2.2		-		0.86
eggs	0.079-0.085 x 0.049-0.054			-	0.04-0.06 x 0.04		0.064 x 0.036	
Type of body segmentation	slightly segmented		no information		ringed and complete segmentation with union in dorsal and ventral region		ringed form and no complete segmentation	
Author	Sarmiento, 1954		Tadros, 1966		Antonio, 1958		present study	
Geographic distribuition	Junin, Peru; Colombia		Rhodesia, South Africa		Venezuela		São Paulo, Brazil	
Vertebrate Host	*Metachirus nudicaudatus*		Baboon		*Tamandua tetradactyla*		*Myrmecophaga tridactyla*	
Reference	Sarmiento, 1954; Tantalean et al., 2005		Tadros, 1966		Antonio, 1958		present study	