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[a,b], Clarice Silva Cesário[c], Natalie Olifiers[d], Rita de Cassia Bianchi[c], Arnaldo Maldonado Jr.[a,*], Roberto do Val Vilela[a]

[a] *Laboratório de Biologia e Parasitologia de Mamíferos Silvestre Reservatório, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz. Avenida Brasil, 4365 Manguinhos, Rio de Janeiro, RJ, CEP, 21045-900, Brazil*
[b] *Pós Graduação em Biologia Parasitária, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*
[c] *Laboratório de Ecologia de Mamíferos, Departamento de Biologia Aplicada à Agropecuária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus Jaboticabal, Jaboticabal, SP, CEP 14884-900, Brazil*
[d] *Universidade Veiga de Almeida, Rua Ibituruna, 108, Maracanã, Rio de Janeiro, RJ, CEP 20271-901, Brazil*

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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 for the first time 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 analyses 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.

1. Introduction

The family Gigantorhynchidae Hamann, 1892 is the only family of the order Gigantorhynchida Southwell and Macfie, 1925 and contains two genera: *Mediorhynchus* Van Cleave, 1916 and *Gigantorhynchus* Hamann, 1892 (Amin, 2013). The genus *Gigantorhynchus* was validated by Yamaguti (1963) and Amin (1985, 2013), and comprises six valid species: *G. 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 baboons in Africa (Tadros, 1966; Amin, 2013). In particular, *G. echinosdiscus* is distributed in the Neotropical region and has been reported parasitizing anteaters in Brazil (Travassos, 1917; Machado Filho, 1941), Venezuela (Días-Ungria, 1958), Panamá (Dunn, 1934), and Trinidad Island (Camerón, 1939).

In Brazil, two species of *Gigantorhynchus* have been reported, *G. lutzi* from the bare-tailed woolly opossum *Caluromys philander* Linnaeus, 1758 (see Machado Filho, 1941), and *G. echinodiscus* infecting anteaters, such 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). Eggs of *G. echinodiscus* were

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* Corresponding author. + 55 21 25621644
E-mail addresses: apngomes@yahoo.com.br (A.P. Nascimento Gomes), clarice86cesario@gmail.com (C.S. Cesário), natolifiers@gmail.com (N. Olifiers), ritacbianchi@gmail.com (R. de Cassia Bianchi), maldonad@ioc.fiocruz.br (A. Maldonado), roberto.vilela@ioc.fiocruz.br (R.d.V. Vilela).

observed in coprolites of *T. tetradactyla* and *M. tridactyla* from an archaeological site in Brazil (Ferreira et al., 1989).

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

Therefore, phylogenetic evidence based on the 28S rRNA gene may be helpful to complement data from conventional taxonomic studies of different taxa.

In the present study, we redescribe *G. echinodiscus* by light and scanning electron microscopy (SEM) and contribute with new molecular data and a phylogenetic approach to the family Gigantorhynchidae.

2. Material and methods

2.1. Specimens collection

The giant anteater *M. tridactyla* was the subject of an ecological research program conducted by São Paulo State University (UNESP) Jaboticabal Campus (*Universidade Estadual Paulisa* - UNESP/ Jaboticabal) and the Institute for Research and Conservation of Anteaters in Brazil (*Instituto de Pesquisa e Conservação de Tamanduás no Brasil - Projeto Tamanduá*). 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 acanthocephalans 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). Acanthocephalan 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, clarified in 90% phenol and whole-mounted as definitive slides in Canada balsam (modified from Amato, 1985). Mounted specimens were examined using an Axion Scope A1 light microscope (Zeiss, Göttingen, Germany). Drawings were made with the aid of a camera lucida attached to a Nikon Eclipse E200MVR light microscope (Nikon Corporation, Tokyo, Japan). Measurements are in millimeters unless otherwise stated, and are presented as the range followed by the mean in parentheses. The proboscis length was the measurement with small and rootless spines, plus the crown of hooks. We made three length measurements of the hooks with root: from the tip of the hook to the root, total length of the hook (blade 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.

For SEM, two males and two females specimens were dehydrated in an ascending ethanol series, critical point-dried method with CO_2, mounted with silver cellotape on aluminum stubs, and sputter-coated with a 20 nm layer of gold. Samples were examined using a Jeol JSM-6390 LVmicroscope (JEOL, Tokyo, Japan) at an accelerating voltage of 15 kV at the Electron Microscopy Platform of Oswaldo Cruz Institute (*Plataforma de Microscopia Eletrônica Rudolf Barth*/IOC- FIOCRUZ).

2.2. Molecular analyses

For molecular studies, one specimen preserved in 70% ethanol were washed in ultrapure water for 24 h at room temperature. Total genomic DNA was isolated using the QIAamp DNA mini kit according to the manufacturer's protocol (Qiagen, Venlo, 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 forward; 5′-ACCCGCTGAATTTAAGCAT-3′ and D2 reverse; 5′-TGGTCCGTGTTTCAAGAC-3′ (Hassouna et al., 1984 - modified from

Chisholm et al., 2001). PCR amplifications were performed using Promega PCR Master Mix (Promega Corporation, Wisconsin, USA). Reactions were 25 μL following the manufacturer's protocol. The thermal-cycling profile was programmed on an Eppendorf Mastercycler Ep System (Eppendorf, Hamburg, Germany) with an initial denaturation step of 95 °C for 2 min; followed by 40 cycles of amplification at 94 °C for 60 s, annealing at 55 °C for 60 s, extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. PCR products were analyzed after electrophoresis on 1.5% agarose gel using GelRed nucleic acid gel stain (Biotium, California, USA) by visualizing in a UV transilluminator. Successful amplifications were purified using the QIAquick PCR purification kit (Qiagen Ltd., Hilden, Germany) following the manufacturer's protocol. Sequencing reactions using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, 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 product precipitations were conducted at the subunit RPT01A – DNA sequencing platform of the Oswaldo Cruz Institute PDTIS/FIOCRUZ.

Electropherograms of the sequences were assembled into contigs, and manually edited for ambiguities using the software package Geneious 9.1.8 (http://www.geneious.com; Kearse et al., 2012). To assess the phylogenetic relationships of *G. echinodiscus*, a matrix with sequences of representatives of the class Archiacanthocephala retrieved from GenBank dataset was generated. Three families, representing three different orders of archiacanthocephalans, were present in our dataset: Oligacanthorhynchidae, represented by two sequences of the genus *Oligacanthorhynchus* Travassos, 1915, one sequence of the genus *Macracanthorhynchus* Travassos, 1917, and one sequence of *Oncicola* Travassos, 1916; Moniliformidae, represented by sequences of the genus *Moniliformis* Travassos, 1915; and Gigantorhynchidae, represented by one sequence of the genus *Mediorhynchus* and our sequence of *Gigantorhynchus* Hamann, 1892. All of these genera infect mammals, while *Mediorhynchus* Van Cleave, 1916 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 1).

We aligned all sequences using the MAFFT program 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 (Amin, 2013; Maddison and Maddison, 2018).

To assess the quality of the data, we tested for the presence of phylogenetic signals with the permutation test probability (PTP) and applied the G1 tests in the program PAUP 4.0a164 (Swofford, 2003). We also investigated 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 graph of transitions and transversions versus JC69 model genetic distances (Jukes and Cantor, 1969) in DAMBE 7.0.35 (Xia, 2018).

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 the tree bisection and reconnection (TBR) branch-swapping algorithm. Node supports in MP were assessed by nonparametric bootstrap percentages (MP-BP) after 10,000 pseudoreplications. ML was carried out using PhyML 3.0 (Guindon et al., 2010) with tree heuristic search using subtree pruning and regrafting (SPR), with 10 random starting trees and model selection

Table 1
Accession numbers of sequences from GenBank used in our phylogenetic analyze using with 28S rRNA gene.

Class	Family	Species	Acession number	Reference
Archiacanthocephala	Oligacanthorhynchidae	*Oligacanthorhynchus tortuosa* (Leidy, 1850)1	AY210466	Passamaneck and Halanych (2006)
		Oligacanthorhynchus tortuosa 2	KM659327	Lopez-Caballero et al. (2015)
		Macracanthorhynchus ingens (Linstow, 1879)	AY829088	Garcia-Varela and Nadler (2005)
		Oncicola venezuelensis Marteau, 1977	KU521567	Santos et al. (2016)
		Moniliformis moniliformis (Bremser, 1811)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* (Müller, 1776)	AY829101	Garcia-Varela and Nadler (2005)
	Plagiorhynchidae	*Plagiorhynchus cylindraceus* (Goeze, 1782)	AY829102	
Eoacanthocephala	Neoechinorhynchidae	*Neoechinorhynchus saginatus* Van Cleave et Bangham, 1949	AY829091	
		Floridosentis mugilis	AY829111	

by the SMS algorithm (Smart Model Selection) (Lefort et al., 2017) under the Akaike information criterion (AIC). Node supports in ML were assessed by the approximate likelihood-ratio test (aLRT) for branches (Anisimova and Gascuel, 2006) and by nonparametric bootstrap percentages (ML-BP) after 1000 pseudoreplicates. 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 and model selection by the SMS algorithm under the Bayesian information criterion (BIC), with two Markov chain Monte Carlo (MCMC) simulation runs, 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 as sufficient.

3. Results

3.1. 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°. 38,580.

3.2. Redescription of Gigantorhynchus echinodiscus (Diesing, 1851) (Figs. 1–17)

General: Body of medium size, narrow, and pseudo segmented (Fig. 8). Sexual dimorphism present, females larger than males. Proboscis cylindrical (Figs. 1, 7 and 12), similar in both sexes, armed with 18 hooks (Figs. 12 and 14), arranged in two rows of hooks that present a root that bifurcated anterior and posteriorly (Figs. 1, 7 and 14). First row with six robust hooks; second row with 12 hooks in pairs, smaller than first row (Figs. 2 and 14). Measurement of the hooks and 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 measuring 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 spines by a slight space without hooks (Fig. 12). Twenty-one to 23 small-rootless spines arranged in longitudinal rows 0.05–0.08 (0.07) (Figs. 1, 2, 12 and 13). One lateral papilla located in the base of the neck on each side with a slightly elevated border and a central pore (Figs. 1, 13 and 15). After the proboscis, there is a small region without pseudo segmentation in both sexes. Lemnisci long and filiform (Fig. 3).

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,
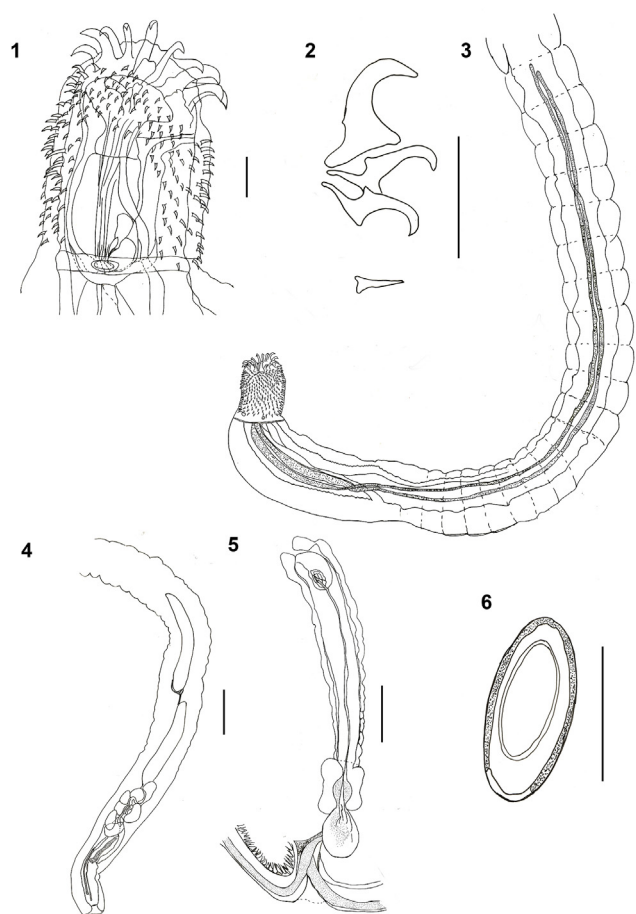
Figs.1–6. Line drawing of *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 1. Praesoma with the proboscis presenting a crown with robust hooks followed by small hooks, a receptacle proboscis, and papillae in the base of the neck; 2. Three different robust hooks in the crown and a one small type in the proboscis; 3. Unsegmented anterior part of the trunk, and lemnisci filiform reaching the middle region of the trunk; 4. Posterior region of adult male showing reproductive organs; 5. Posterior region of adult female showing the uterus, vagina and gonopore subterminal; 6. Egg.

0.30–0.55 (0.45) wide, with 18 apical hooks followed by 21–23 small rootless spines arranged on longitudinal rows. After the proboscis, a region without segmentation, 2.24–3.21 (2.72) long (Fig. 8). Proboscis receptacle 0.48–0.64 (0.57) long, 0.21–0.32 (0.26) wide (Fig. 1). Lemnisci 8.02–20.30 (14.87) (n = 3) long, reaching the middle of the trunk and sometimes bent on themselves (Fig. 8). Two ellipsoid testes, narrow, and in tandem; anterior testis 1.63–2.71(2.25) long, 0.26–0.32
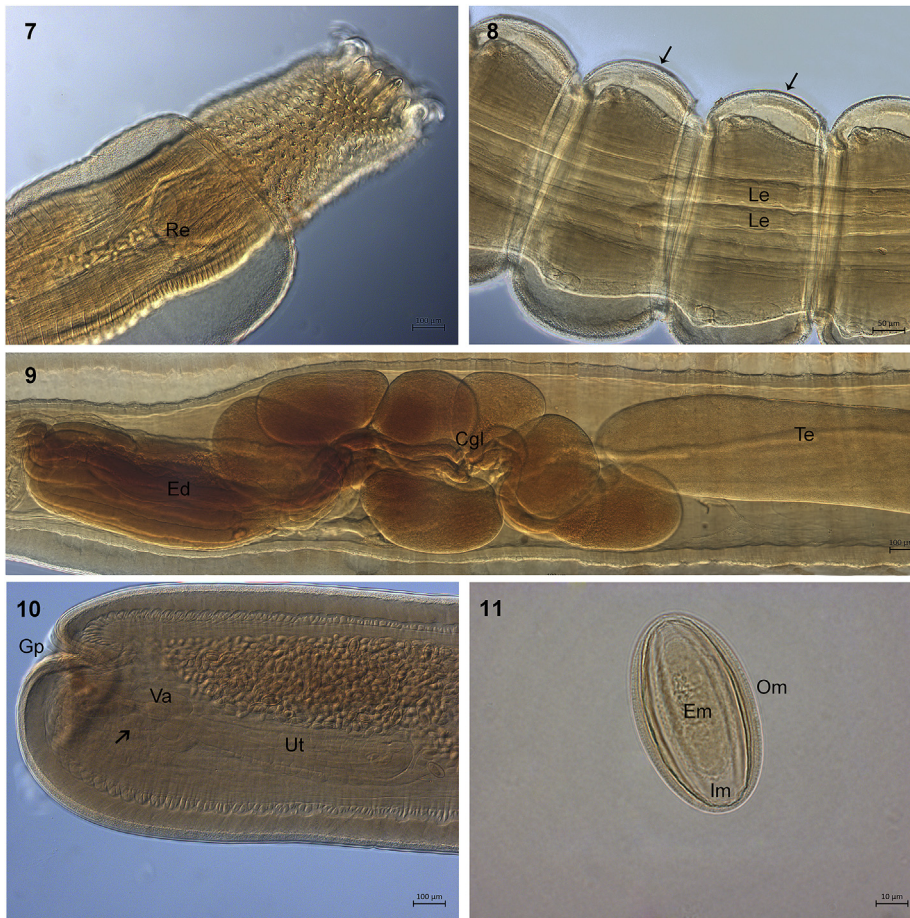
Figs. 7–11. Light microscopy of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 7. Proboscis with a crown of large hooks in the apex and small hooks, and a proboscis receptacle (Re); 8. Trunk with pseudo segmentation (arrows) and the end of the lemnisci (Le); 9. Male reproduction organs, testis (Te), cement glands in pair (Cgl), ejaculatory duct (Ed); 10. Detail of the posterior end of adult female showing the uterus (Ut), vagina (Va, arrow) and gonopore subterminal (Gp); 11. Egg ellipsoid showing the outer membrane thick (Om), inner membrane (Im) thin, embryo (Em).

(0.29) wide; posterior testis 1.61–2.66 (2.13) long, 0.26–0.39 (0.29) wide (Fig. 4). Eight cement glands in pairs, the group measuring 0.98–2.13(1.61) long and 0.45–0.76 (0.60) wide (Figs. 4 and 9), followed by ejaculatory duct, 0.82–1.42 (0.97) long. Posterior end after the anterior testes without a segmentation region and measuring 5.45–8.53 (6.83), with smooth surface and a copulatory bursa at the end (Figs. 4, 9, 16 and 17).

Female (six specimens): Body 102.79–52.92 (75.45) long, 0.79–1.13 (0.85) wide. Proboscis and neck 0.49–0.71 (0.55) long, 0.46–0.53 (0.48) wide. Proboscis receptacle 0.63–0.74 (0.70) long, 0.23–0.31 (0.27) wide (Fig. 1). Lemnisci long, 13.23 mm long (n = 1) (Fig. 8). Gonopore subterminal and vagina has sinuous lateral region in "guitar" format (Figs. 5 and 10). Uterine bell to genital pore including the vagina, uterus, and uterine bell 0.69–0.97 (0.86) long (n = 5) (Fig. 5). Eggs ellipsoid, with three membranes 0.059–0.069 (0.064) long, 0.04–0.03 (0.036) wide (n = 26; Figs. 6 and 11).

3.3. Molecular analyses

Sequencing resulted in a partial 28S rRNA gene consensus sequence of 771bp from one adult *G. 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 G1 (G1 = 0.9227) tests indicated the presence a 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 step 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 in PhyML under AIC was 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 in PhyML was HKY + G and the BI resulted in a mean estimated marginal likelihood of −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 Fig. 18. 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), although with low support, 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 suggested it is a 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.

3.4. Remarks

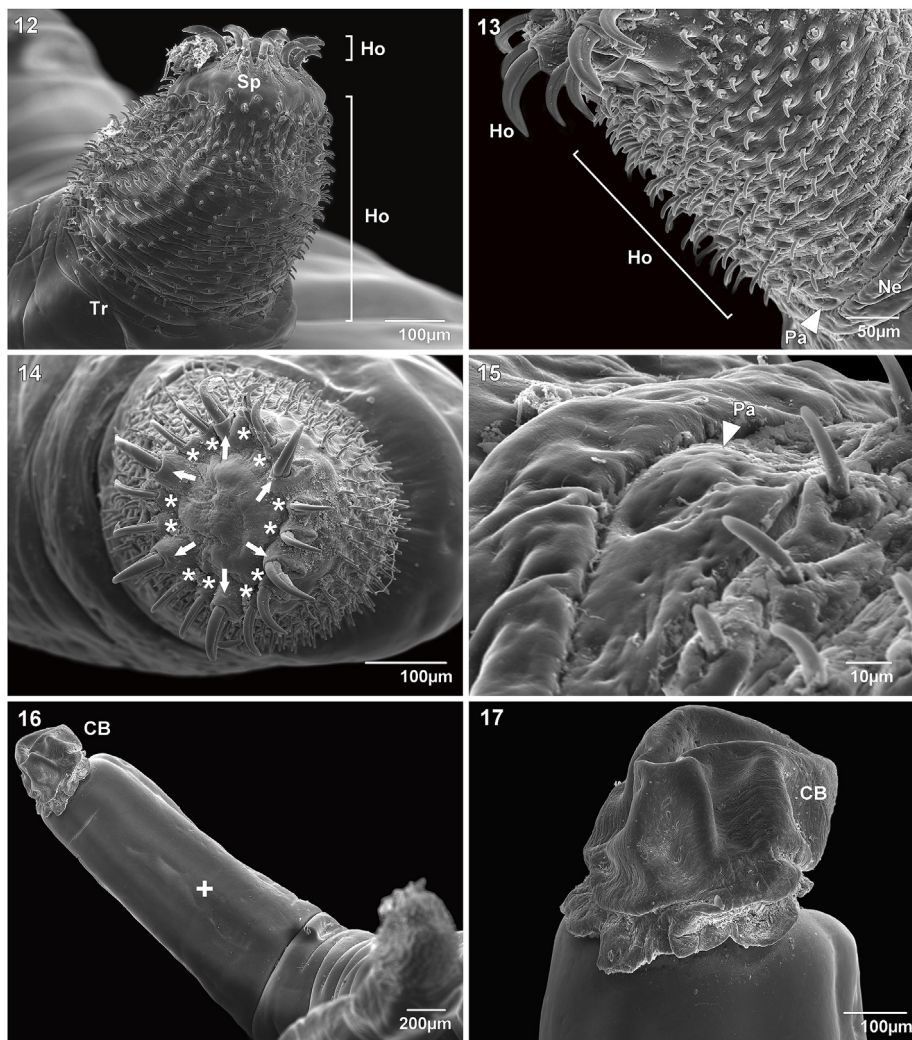Species of the genus *Gigantorhynchus* are characterized by the

Fig. 12–17. Scanning electron micrographs of adult *Gigantorhynchus echinodiscus* from *Mymercophaga tridactyla*. 12 and 13. Cylindrical proboscis armed with hooks (Ho) showing a space (Sp) between the two circles of large hooks and small rootless spines, neck (Ne), trunk (Tr), lateral papillae (Pa, arrowhead); 14. Detail of the crown with two circles of large hooks (arrow – 1st row and asterisk – 2nd row); 15. Detail of the lateral papillae; 16 and 17. Posterior end of adult male showing the region without pseudo-segmentation (cross) and a copulatory bursa protruding from the body (CB).

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; Southwell 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 (Tadros, 1966) (Table 2). The taxonomy of this species needs to be revised.

The specimens we found parasitizing *M. tridactyla*, were identified as *G. echinodiscus* 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 2).

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 (Table 2).

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 (suplemenntary data).

4. Discussion

The genus *Gigantorhynchus* was erected by Hamann (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 into 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),
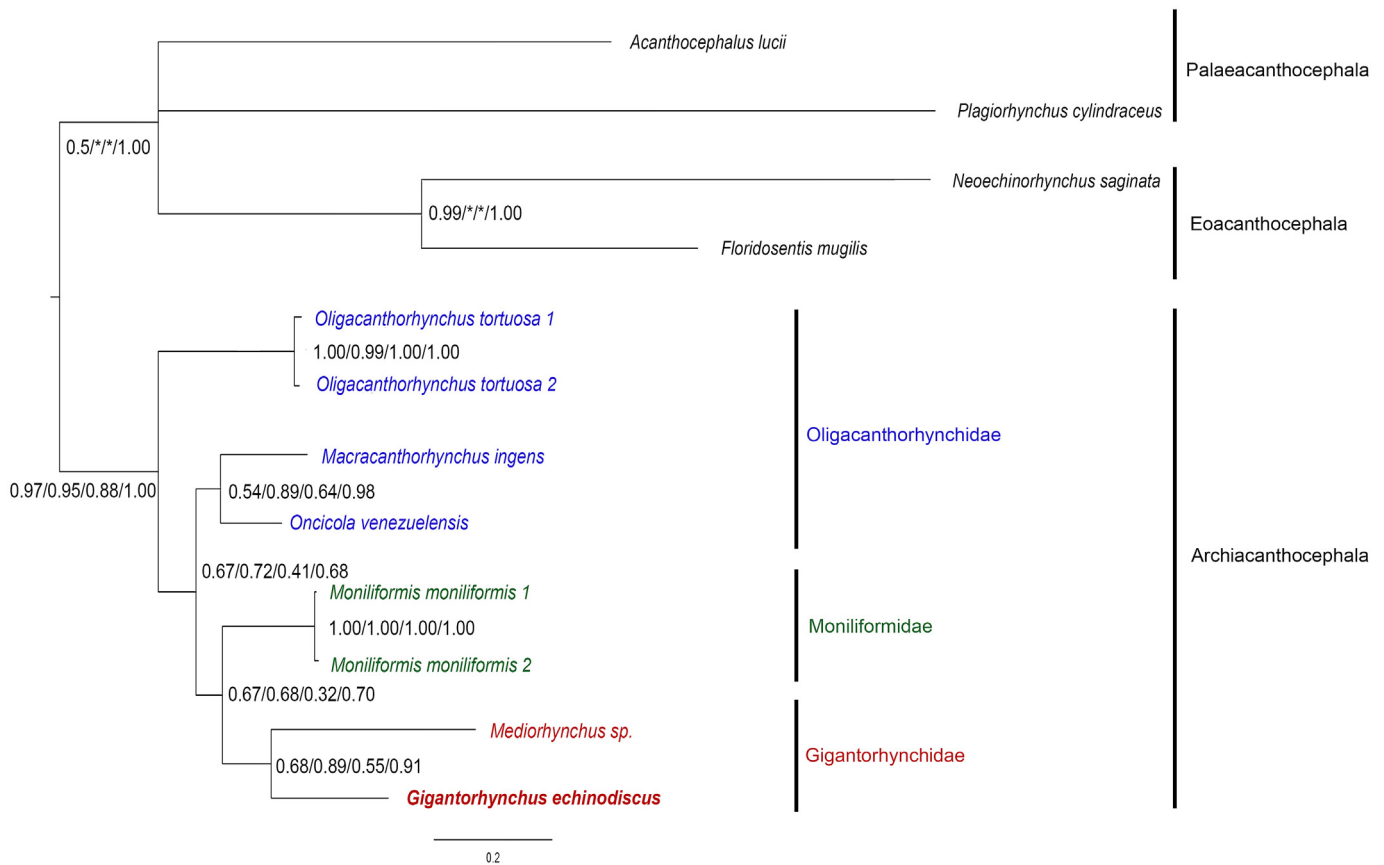
Fig. 18. Bayesian inference phylogenetic reconstruction tree of 28S rRNA gene sequences of *G. echinodicus* in the present study (in red and bold) and archiacanthocephalans sequences from GenBank. The class Palaeacanthocephala, and Eoacanthocephala were added as outgroups. Node values are MP-BP, aLRT, ML-BP, and BPP, respectively.* no support or node not recovered in the respective analysis. Blue – family Oligacanthorhynchidae; green – family Moniliformidae; red – family Gigantorhynchidae.

parasites of mammals and birds. Van Cleave (1923) reviewed Acanthocephala, proposing a classification key to the genera considered valid, including the genus *Gigantorhynchus*, which includes parasites of mammals from the Neotropical region. Later, Southwell and Macfie (1925) divided Acanthocephala into 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), analyzing 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 24 more species in the genus *Gigantorhynchus* as synonyms of different genera. Amin (2013) recently updated the classification of the family Gigantorhynchidae including two genera: *Gigantorhynchus* and *Mediorhynchus,* in agreement with Van Cleave (1953).

Amato et al. (2014) reported, for the first time in Brazil, cystacanths of *G. echinodiscus* infecting termites as intermediate hosts. The giant anteater's diet consists almost exclusively of termites (Rodrigues et al.,

2008; Gaudin et al., 2018), suggesting that these arthropods are intermediate hosts of *G. echinosdiscus*.

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 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.

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 cluster these two genera within the family Gigantorhynchidae.

Furthermore, our phylogenetic analyses of the class Archiacanthocephala genera agree with previous studies, indicating that 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 *Moniliformis* (García-Varela and Nadler, 2005; Amin et al., 2013; García-Varela and Pérez-Ponce de León, 2015; Amin et al., 2016). Of particular note was the basal, non-monoplyletic Oligacanthorhynchidae, suggesting that relationships may not be well resolved within this group, and the characters distinguishing this group may be plesiomorphic, requiring more thorough studies.

In conclusion, our 28S rRNA gene study provides the first DNA

Table 2
Morphometric comparisons of *Gigantorhynchus* species (measurements in millimeters).

Species	*Gigantorhynchus lutzi*		*Gigantorhynchus lopezneyrai*		*Gigantorhynchus ortizi*	
Sex	Male	Female	Male	Female	Male	Female
Trunk Length	35–60	130–200	16–58	–	46–75	130–242
Trunk Width	0.75–1.15	1–2.5	1–1.7	–	1.4–1.92	1.5–2.0
Anterior end without segmentation	–		no region without segmentation			
Proboscis Length	1.695		1.131–1.5		1.45–1.72	
Proboscis Width	0.735		0.66		0.435–0.555	
Number of hooks	12 (6 + 6)		12 (4 + 8)		12 (6 + 6)	
Hook to root x root	0.285 × 0.165 (1st row), 0.225 × 0.135 (2nd row)		0.235 (1st row), 0.106 (2nd row)		0.160 × 0.10 (1st row), 0.140 × 0.09 (2nd row)	
Small rootless spines length	0.048		–		0.05	
Receptacle	–		–		0.750–0.920	
Lemnisci	2.595		8		5.48–6.80	
Anterior testis	5.752–6.045 × 0.750–0.900		0.7 × 0.190		1.98–3.0 × 0.56–0.96	
Posterior testis						
Number of cement glands	8		8		8	
Dimension group of cement glands	–		–		–	
Organization of cement glands	in pairs		in pairs		in group	
Ejaculatory duct	2.10–2.55		–			–
uterine bell	1.575 × 0.270		–			–
eggs	0.115 × 0.064		–		0.079–0.085 × 0.049–0.054	
Type of body segmentation	ringed form and no complete segmentation		slightly segmented		slightly segmented	
Author	Machado Filho (1941)		Díaz-Ungría (1958)		Sarmiento (1954)	
Geographic distribution	Pará, Brazil; Huanuco, Peru		Venezuela		Junin, Peru; Colombia	
Vertebrate Host	*Caluromys philander; Didelphis marsupialis*		*Tamandua tetradactyla*		*Metachirus nudicaudatus*	
Reference	Machado Filho (1941); Tantalean et al., 2005		Díaz-Ungría (1958)		Sarmiento (1954); Tantalean et al., 2005	

Species	*Gigantorhynchus pesteri*		*Gigantorhynchus ungriai*		*Giganthorhynchus echinodiscus* (present study)	
Sex	Male	Female (immature)	Male	Female	Male	Female
Trunk Length	–	15–18	22–36	129–136	31.53	75.45
Trunk Width	–	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 Length	0.35		0.189–1.0		0.50	0.55
Proboscis Width	0.1		0.237–0.7		0.30–0.52 (0.42)	0.48
Number of hooks	4		18 (6 + 12)		18 (6 + 12)	
Hook to root x root	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 rootles spines length	0.015		0.02–0.06		0.07	
Receptacle	0.75 × 0.18–0.2		–		0.57 × 0.26	0.70 × 0.27
Lemnisci	3.6–4		1.75–3.27		14.87	
Anterior testis	–		2.0–5.6 × 0.395–0.474		2.25 × 0.29	
Posterior testis					2.13 × 0.29	
Number of cement glands	–		8		8	
Dimension group of cement glands	–		0.869 × 0.1896		1.61 × 0.60	
Organization of cement glands	–		–		in pairs	
Ejaculatory duct	–		2.6		0.97	
uterine bell		2.2	in group	–		0.86
eggs	–		0.04–0.06 × 0.04		0.064 × 0.036	
Type of body segmentation	no information		ringed and complete segmentation with union in dorsal and ventral region		ringed form and no complete segmentation	
Author	Tadros (1966)		Antonio (1958)		present study	
Geographic distribution	Rhodesia, South Africa		Venezuela		São Paulo, Brazil	
Vertebrate Host	Baboon		*Tamandua tetradactyla*		*Myrmecophaga tridactyla*	
Reference	Tadros (1966)		Antonio (1958)		present study	

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.

Declaration of competing interest

On behalf of the authors, all the authors disclose any financial interest, personal relationship with other people or organizations and commercial sponsor for this work which could inappropriately influence the work and causes conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijppaw.2019.09.008.

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