ABSTRACT

Nematodes from the genus *Helminthoxys* Freitas, Lent & Almeida, 1937 are intestinal parasites of caviomorph rodents with a wide Neotropical distribution. This study detailed the morphology of *Helminthoxys freitasi* Quentin, 1969 using light microscopy and scanning electron microscopy (SEM), and inferred a phylogeny for the tribe Protozoophagini using partial mitochondrial cytochrome c oxidase subunit I gene sequences (MT-CO1). Rodents *Mesomys hispidus* (Desmarest, 1817) were collected in three distinct areas in the state of Acre, Brazil. The helminths were recovered and morphology of their surfaces, such as lateral alae reaching the level of the anus, the posterior region of the body in the male having three pair of sessile papillae and one pair papillae pedunculated were detailed. Genetic sequence of *H. freitasi* suggested a close relationship with the genus *Wellcomia* Sambon, 1907, corroborating a previous morphological phylogeny. A new host species, *M. hispidus*, and a new locality in the Amazon rainforest is recorded.

*Keywords*: Cytochrome c subunit I – Integrative Taxonomy – *Mesomys hispidus* – Scanning Electron Microscopy – Syphaciinae
INTRODUCTION

The genus *Helminthoxys* Freitas, Almeida and Lent, 1937 currently comprises eight species. The type species *Helminthoxys caudatus* (syn. *H. pujoli* Quentin, 1973) was first described infecting the rodent *Microcavia australis* in Argentina (Freitas-Texeira *et al.*, 1937). Subsequently other species were described: *H. tiflophila* Viguera, 1943 in *Mysateles prehensilis* (Viguera, 1943); *H. effilatus* Schuurmans-Stekhoven, 1951 (syn. *H. velizi* Parra Ormeño, 1953) in *Lagidium viscacia* (Schuurmans-Stekhoven, 1951); *H. urichi* Cameron & Reesal, 1951 in *Dasyprocta leporina* (Cameron & Reesal, 1951); *H. quintini* Barus, 1972 in *Capromys pillorides* (Barus, 1972); *H. gigantea* Quentin, Courtin & Fontecilla 1975 in *Octodon degus* (Quentin, Courtin & Fontecilla 1975); *H. freitasi* Quentin, 1969 in *Trichomys laurentius* (syn. *Trichomys apereoides*) (Quentin, 1969); and *H. abrocameae* Hugot & Gardner, 2000 in *Abrocoma cinerea* (Hugot & Gardner, 2000).

These nematodes inhabit the large intestine of caviomorph rodents of seven different families, which include the following: Caviidea, Capromyidae, Chinchiliidae, Dasyproctidae, Echimyidae, Octodontidae, and Abrocomidae (Hugot, 1988).

Studies on the morphologic phylogeny of the order Oxyurida based on morphologic characters of the reproductive structures of the male and cephalic plate, proposed that the tribe Protozoophagini is composed of three genera which include the following: *Helminthoxys* Freitas, Lent & Almeida, 1937; *Wellcomia* Sambon, 1907; and *Protozoophaga* Travassos, 1923 (Hugot, 1988). Later studies based on molecular phylogenetic inference have confirmed the evolutionary relationship between *Wellcomia* Sambon, 1907 and *Protozoophaga* Travassos, 1923 (Nadler *et al.*, 2007), but not included *Helminthoxys* Freitas, Almeida & Lent, 1937. Nevertheless, so far, no molecular phylogeny has included molecular sequence *Helminthoxys* and representatives of the sister Syphaciini tribe.

This study detailed the morphology of *Helminthoxys freitasi* Quentin, 1969 by light microscopy and scanning electron microscopy (SEM), adding further taxonomic characteristics for species and inferred a phylogeny for the tribe Protozoophagini using partial mitochondrial cytochrome *c* oxidase sub unit I gene (MT-CO1). In addition, both a new host species and new geographic locality were recorded.

MATERIALS AND METHODS

Collection sites

This study was developed in three distinct areas within the Amazon rainforest in the state of Acre, in the municipalities of Porto Acre (9°54’17.70"S; 67°17’8.01"W), Senador Guiomard (10°09’39.0"S; 67°44’17.6"W), and Xapuri (10°49’40.79"S; 68°21’38.89"W). Rodents were...
trapped using Tomahawk (model 201, Hazelhurst, Wisconsin) and Sherman (model XLK, H.B. Sherman Traps, Tallahassee, Florida) live traps. To capture arboreal mammals, traps were tied to tree branches placed in the forest understory. Captures occurred during five consecutive nights in 2014, 2015, and 2016. Euthanasia followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research and the Brazilian Guide to Good Practices for Euthanasia in Animals (Sikes, 2016). Permits for rodent capture and handling were issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), and experimental procedures on animals were approved by the Ethics Committee on Animal Use (CEUA) of the Instituto Oswaldo Cruz.

Helminths collection

After collection, worms were washed in saline, sodium chloride solution (NaCl 0.9%) and maintained in 70% ethanol solution. For examination under light microscopy, nematodes were clarified in lactophenol 90% and drawings were produced with aid of a Camera Lucida, attached to a Zeiss Scope Z1 light microscope (Zeiss, Göttingen, Germany). All measurements were in micrometers. The structures were measured through digital images captured by a Zeiss Axio Cam HRC (Zeiss, Germany) using the accessory software Axio Vision Rel. 4.7 (2009).

For SEM analyses, six specimens (three males and three females) of post-fixed helminths were dehydrated in increasing ethanolic series (70%, 80%, 90%, and absolute ethanol), for 20 min at each stage, and dried by the critical point method with CO2 (Souza et al., 2017). The samples were then submitted to gold metallization with layer thickness of approximately 20nm. The specimens were then analyzed in a SEM JEOL JSM6390LV at the Plataforma de Microscopia Eletrônica Rudolf Barth, Instituto Oswaldo Cruz, Fiocruz (Electron Microscopy Platform of the Oswaldo Cruz Institute).

A paratype of H. freitasi from the Coleção Helmintológica do Instituto Oswaldo Cruz – CHIOC (Nº 30936) was used to compare morphological characteristics. Voucher specimens were deposited in CHIOC under the following number: CHIOC 38502.

Molecular and phylogenetic analyses

Genomic DNA was isolated from three individual pinworms from the Senador Guiomard and Porto Acre localities using the QIAamp DNA Mini Kit, applying the manufacturer’s protocol (QIAGEN, Hilden, Germany).

DNA amplification by polymerase chain reaction (PCR) was conducted using the following primers: SyphaCO1_F (5’- ACCCGCTGAATTTAAGCAT-3’) and SyphaCO1_R (5’- ACCAACCCTAAACATAAA-3’) to produce amplicons of the mitochondrial cytochrome c oxidase subunit I gene (MT-CO1) fragments (Okamoto et al., 2007).

Each PCR contained 1x PCR buffer, 4 mM MgCl2, 0.2 µM of each primer, 0.2 mM of each deoxynucleotide triphosphate solution (dNTPs), 1U of Platinum TaqDNA polymerase (Invitrogen, São Paulo, Brazil), 2.0 µL of genomic DNA, and ultrapure water, in a total reaction volume of 25 µL. PCR-cycling parameters followed Okamoto et al., (2007). Resulting amplicons were visualized on 1.5% agarose gels after electrophoresis, using Gel Red™ nucleic acid gel stains (Biotium, Hayward, California, USA), and UV transilluminator. Successfully amplified amplicons were purified using the Illustra™ GFX™ PCR DNA and Gel Band Purification Kit following the manufacturer's protocol (GE Healthcare, Little Chalfont, UK). Amplicons were cycle-sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on both strands using the PCR primers mentioned, resulting in bidirectional sequencing for better data accuracy. Sequencing was performed using the ABI3730 DNA Analyzer. Both procedures and cycle-sequenced products precipitation were conducted at the Plataforma de Sequenciamento de DNA do Instituto Oswaldo Cruz, PDTIS/Fiocruz (DNA Sequencing Platform of the Oswaldo Cruz Institute). Fragments were assembled into contigs and edited for ambiguities using the software Geneious 9.1.8 (Kearse et al., 2012), resulting in consensus sequences.

Our dataset included sequences from the closest relatives of Helminthoxys genus, oxyurids of the subfamily Syphaciinae. These oxyurids belong to four genera each representing a different tribe as follows: Passalurus Dujardin, 1845;
Rauschtineria Hugot, 1980; Syphacia Seurat, 1916, and Wellcomia Sambon, 1907. We also included sequences of genera Enterobius Leach, 1853 and Lemuricola Chabaud et Petter, 1959 as a representative of the oxyurid subfamily Enterobinae. The oxyurid Aspiculuris tetraptera Schulz, 1924, from the family Heteroxynematidae, was included as outgroup. The subfamilies, tribes of Syphacinae, species, GenBank accession numbers, and references of specimens used in this study are listed in Table 1.

We aligned the MT-CO1 sequences using the Translator X online software (Abascal et al., 2011). Resulting alignments were trimmed of poorly aligned regions using the Mesquite package software (Maddison & Maddison, 2011). Substitution saturation in the dataset was assessed via the Test by Xia (Xia et al., 2003; Xia & Lemey, 2009) using the DAMBE program, Version 6.4.79 (Xia & Xie, 2001).

Phylogenetic reconstructions using maximum likelihood (ML) were carried out using PhyML 3.0 software (Guindon et al., 2010). Nucleotide evolutionary model selection was executed with SMS (Smart Model Selection) (Lefort et al., 2017) in PhyML, using the Bayesian information criterion (BIC). Node support in ML trees was assessed by the Approximate Likelihood-Ratio Test for Branches (aLRT) (Anisimova & Gascuel, 2006) and by nonparametric bootstrap percentages (ML-BP) after 1000 pseudoreplications. Bayesian phylogenetic inference (BI) was carried out using the MrBayes program, version 3.2.6 (Ronquist et al., 2012) on XSEDE using the CIPRES Science Gateway (Miller et al., 2010). To account for different evolutionary processes at each of the three codon positions, BI analyses were performed using the GTR+G model for each codon position, with unlinked base frequencies and parameters. Markov chain Monte Carlo samplings were performed for 10,000,000 generations with four simultaneous chains in two runs. The robustness of nodes was assessed by Bayesian posterior probabilities (Bpp) calculated from tree samples every 100 generations, after removal of a “burn-in” fraction of 25%. To assess the level of variation in the (COI) among the selected samples of different taxa, uncorrected (p) pairwise genetic distances were calculated using PAUP* 4.0b10 software (Swofford, 2002).

Ethical standards
License for animal capture was provided by the Instituto Chico Mendes de Conservação da Biodiversidade- ICMBio (permanent license 13373-1). All protocols followed the guidelines for capture, handling and care of the Ethics Committee on Animal Use of the Oswaldo Cruz Institute (according to license L-049/08) (protocol P-70/13-2; license LW-39/14).

Conflict of interest
The authors declare no conflict of interest.

RESULTS

Morphology by scanning electron and light microscopy
Adult helminths exhibited sexual dimorphism. In both sexes (Figures 1A and 3A) the anterior extremity had three prominent pseudolabia, one ventral and two dorsolateral, interspersed with three strong conical esophageal teeth (Figure 3B), which were intercalated with cuticularized thickenings of the inner part of the pseudolabia and the vestibule (Figure 1B). In the external part, surrounded by a rough cuticular area located on each dorsolateral pseudolabia, two labial papillae were closely grouped laterally with corresponding amphids (Figure 1C). Morphological analysis by scanning electron microscopy showed the cuticular expansions which formed the cervical alae extend in lateral alae reaching the level of the anus, in the light microscope only the cervical wing was clearly seen (Figure 3A).

Males (Figures 1A and 2A) had two cuticular mamelons protruding as cuticular expansions with longitudinal ridges located in the posterior part of the body (Figures 2C and 3F). There was 18 ventral trimmings after the second mamelon in the form of small longitudinal cuticular ridges (Figures 2B and 3E), one long spicule, gubernaculum, accessory
Figure 1. *Helminthoxys freitasi* A) Complete male view; B) Head, left lateral view; C) En face view of head; D) Ventral view of caudal bursa showing one pair of sessile papillae and one pair of pedunculate papillae; E) Cross-section of the body at the level of the cervical alae; F) Cross-section of the body at the level of the first mamelon; G) Cross-section of the body at the level of the area rugosa posterior to the second mamelon; H) Area rugosa posterior to the second mamelon, ventral view; I) Uterus didelphic and a pair of spermatheca (arrow). Scale: A, E, F, G, H, I, 50µm; B, C, D, 10µm.
Figure 2. *Helminthoxys freitasi* A) Adult male, general view; B) Bursal caudal, detail of the area rugosa posterior to second mamelon (asterisk); C) First and second mamelon (arrow), lateral view; D) Detail, spicule (S) and gubernaculum (arrow), lateral view; E) Detail, vulva (large arrow), lateral view; F-G) Eggs. *Scale:* A, 100µm; B, C, D, E, 50µm; F, 10µm.
hooks at the base of the cloacal opening, three pair of sessile ad-cloacal papillae, and one pair of pedunculate posterior papillae (Figures 1D, 1H, 2D, 3C, and 3D). Phasmids were located anteriorly to the pedunculate pair of papillae.

In the females, the vulva was in the posterior part of the body (Figure 2E). The uterus folded on itself, opening in two oviducts with a pair of spermatheca (Figure 1I). Eggs were asymmetrical and not operculated (Figure 2F).

Morphometric data including all the species of the genus Helminthoxys, from their original descriptions were compared, emphasizing distinctions of our specimen and added new data with the measurements of eggs not previously described (listed in Table 2).

**Taxonomic Summary**

Host: *Mesomys hispidus* (Desmarest, 1817) (Rodentia: Echimyidae).

Site infection: large intestine

Locality: Municipalities of Porto Acre (9°54’17.70”S; 67°17’8.01”W), Senador Guimard (10º09’39.0”S;67º44’17.6”W), and Xapuri (10°49’40.79”S; 68°21’38.89”W), State of Acre, Brazil.

Mean intensity: 6.2 (31 specimens out of 5 host infected)

Prevalence: 45.4 (5 host infected out of 11 host examined)

Abundance: 2.81 (31 specimens out of 11 host examined).

Specimens deposited: CHIOC Nº 38502

**Molecular and phylogenetic analyses**

We obtained consensus MT-CO1 sequences from three adult *Helminthoxys freitasi* recovered from two hosts from different localities. Two consensus sequences were obtained from Porto Acre (A) and one was obtained from Senador Guimard (B). The sequence from A were identical 975 bp whereas the sequence from B had 954 bp and differed from A by a single transition, representing two distinct MT-CO1 haplotypes. Both sequences were deposited in the GenBank database under accession numbers: MH212135 and MH212136.

The resulting aligned matrix with GenBank sequences comprised of 18 taxa (shown in Table 1) and 819 characters, of which 482 characters were constant, 118 variable characters were parsimony-uninformative, and 201 were parsimony informative. The test by Xia (Xia et al., 2003; Xia & Lemey, 2009) provided evidence for substantial saturation only at the third codon positions, whereas at the first and second positions, and overall there was little saturation in the matrix.

As the best-fit model, PhyML-SMS selected the GTR+G model nucleotide substitution, with ML optimized frequencies, estimated Gamma-shape parameter (α=0.280), and four rate categories. The best log-likelihood ML tree score was -4469.546140.

For the BI, the mean estimated marginal likelihood was -4077.7366 and the median was -4077.421. ESSs for all parameters were above 1000 effectively independent samples and for most parameters, indicating the robustness of our sampling.

The pairwise uncorrected *p*-distances for representatives of tribes of the subfamily Syphaciinae and subfamily Enterobiinae are summarized in Supplementary Table S1. Overall, our matrix had pairwise genetic intraspecific *p*-distances from 0.1% to *Helminthoxys* and to *Passarulus* genera and 17.9% interspecific distances between *W. siamensis* Nadler, 2007 and *E. vermicularis* (Linneu, 1758) Leach, 1853 (mean = 13.1%). The genetic distance between Syphaciinae and Enterobiinae ranged from 11.6% between *R. eutamii* (Tiner, 1948) and *E. macaci* Yen, 1973, to 17.9% between *P. ambiguus* Rudophi, 1819 and *E. vermicularis* (mean = 14.5%).

The genetic distance between *H. freitasi* and *W. siamensis* (i.e. within the tribe Protozoophagini) ranged from 11.5–11.6% (mean=11.5%). The distance between Protozoophagini and Hilgertini ranged from 12.7% between *H. freitasi* and *R. eutamii*, to 16.5% between *W. siamensis* and *R. eutamii* (mean = 14.3%). The distances between Protozoophagini and Syphaciini ranged from 12.7% between *H. freitasi* and *S. stroma*, to 17.5% between *W. siamensis* and *S. agrariani* (mean = 14.7%). The distances between Protozoophagini and Passalurini ranged from 12.7% between *H. freitasi* and *P. ambiguus*, to 15.3% between *W. siamensis* and *P. ambiguous* (mean = 13.7%).

Neotropical Helminthology, 2019, 13(2), jul-dic

Mitochondrial DNA and morphology data of *Helminthoxys*
ML and BI phylogenies resulted in similar topologies with little variation in nodes and support values, as shown in Figure 4. All analyses agreed with *H. freitasi* haplotypes forming a monophyletic group, sister to *W. siamensis* with strong support (aLRT=99%, BP-ML=100%, BPP=100%). The tribes Hilgertiini, Protozoophagini, and Syphaciini formed a monophyletic group with strong support only in the aLRT (99%) and the BPP (99%). Passalurini was a sister group to the other tribes. The subfamily Syphaciinae thus formed a monophyletic group, including all four tribes represented in our sample, although with weak to moderate support (aLRT = 89%, BP-ML = 51%, BPP = 63%). The subfamily Enterobiinae also formed a monophyletic group, although with weak to moderate support (aLRT = 77%, BP-ML = 39%, BPP = 62%).

### Table 1

| Subfamily      | Syphaciinae Tribes | Species              | Genbank accession number | Reference                  |
|----------------|--------------------|----------------------|--------------------------|----------------------------|
| Heteroxynematinae |                   | *Aspiculuris tetraptera* | KT764937                 | Wang *et al.* (2016)       |
| Enterobiinae    |                    | *Enterobius macae*    | AB626858                 | Hasegawa *et al.* (2012)   |
|                 |                    | *Enterobius vermicularis* | EU281143               | Kang *et al.* (2016)       |
| Syphaciinae     | Passalurini        | *Passarulus ambiguus* | KT879302                 | Liu *et al.* (2016)        |
|                 |                    | *Passarulus ambiguus* | KF472059                 | Sheng *et al.* (2014)      |
|                 | Hilgertiini        | *Rauschtineria eutamii* | KT875323                | Bell *et al.* (2016)       |
|                 |                    | *Rauschtineria eutamii* | KT875241                | Bell *et al.* (2016)       |
|                 | Shypaciini         | *Syphacia frederici*  | MF142425                 | Stewart *et al.* (2016)    |
|                 |                    | *Syphacia montana*    | AB282581                 | Okamoto *et al.* (2007)    |
|                 |                    | *Syphacia obvelata*   | KT900946                 | Wang *et al.* (2016)       |
|                 |                    | *Syphacia agraria*    | AB282589                 | Okamoto *et al.* (2007)    |
|                 | Protozoophagini    | *Helminthoxys freitasi A* | MF212135             | This study                 |
|                 |                    | *Helminthoxys freitasi B* | MF212136             | This study                 |
|                 |                    | *Wellcemia siamensis* | GQ332427                 | Park *et al.* (2011)       |
Table 2 - Measurements, in micrometers, of male and female of all species of genus *Helminthoxys*, plus the specimens in study.

|               | Male                             | H. caudatus (H. pujoli) | H. tifilophila (H. velizy) | H. effilatus | H. urichi | H. quentini | H. gigantea | H. abrocomae | H. freitasi | H. freitasi |
|---------------|----------------------------------|--------------------------|-----------------------------|--------------|-----------|-------------|-------------|---------------|-------------|-------------|
| Body length (L) | 5.500                            | 6.550                    | 6.800                       | 3.000        | 5.240     | 6.320       | 11.672      | 5.030         | 3.725       |
| Body width (W)  | 330                              | 340                      | 360                         | 200          | -         | -           | 367         | 230           | 230         |
| Nervous ring    | 190                              | 240                      | 200                         | 120          | 200       | 180         | 306         | 170           | 183         |
| Excretory pore  | -                                | 1.400                    | 1.250                       | 900          | 1.200     | 1.200       | 1.151       | 930           | 840         |
| Oesophagus (L)  | 660                              | 860                      | 800                         | 350          | 730       | 700         | 957         | 370           | 546         |
| Bulb (L x W)    | 170x160                          | 200x150                  | 290x200                     | 130x110      | 220x160   | 200x115     | 285x153     | 150x120       | 172x124     |
| Tail (L)        | 930                              | 650                      | 550                         | 410          | 490       | 900         | 1.365       | 475           | 390         |
| Tip of tail (L) | 830                              | -                       | 420                         | 350          | -         | 850         | 1.243       | 430           | 319         |
| 1st mamilon to tip tail | 3.100                             | -                       | 3.400                       | -            | -         | 3.400       | 5.938       | 2.450         | 2.325       |
| 2nd mamilon to tip tail | 3.500                              | -                       | 3.880                       | -            | -         | 3.900       | 7.099       | 2.730         | 2.565       |
| Spicule         | 320                              | 420                      | 295                         | 533* Hugot, 1986 | 175      | 234         | 866         | 630           | 580         |
| Gubernaculum    | 45                               | 120                      | 50                          | 45           | 38        | 80          | 152         | 60            | 42.5        |
| Body size/spicules (%) | 5.8                             | 6.4                      | 4.3                         | 17.6         | 3.3       | 3.7         | 7.4         | 12.5          | 15.5        |

| Female          |                                 |                          |                             |              |           |             |             |               |             |
| Body length (L) | 11.620                           | 19.200                   | 20.660                      | 8.640        | 12.480    | 13.500      | 21.211      | 13.000        | 10.325      |
| Body width (W)  | 530                              | 800                      | 800                         | 600          | 790       | 420         | 654         | 525           | 407         |
| Nervous ring    | 270                              | 200                      | 400                         | 110          | 270       | 270         | 512         | 215           | 249         |
| Excretory pore  | -                                | 2.250                    | 2.470                       | 1.400        | 1.170     | 1.830       | 3.023       | 1.700         | 1.200       |
| Oesophagus (L)  | 710                              | 1.250                    | 1.300                       | 580          | 990       | 1.150       | 1.383       | 650           | 697         |
| Bulb (L x W)    | 250x190                          | 350x-                    | 380x250                     | 180x180      | 290x270   | 270x180     | 359x205     | 225x200       | 219x179     |
| Tail (L)        | 1.150                            | 1.600                    | 3.130                       | 1.220        | 1.310     | 1.900       | 3.381       | 1.720         | 562         |
| Vulva to tip tail | 4.160                          | 8.000                    | 7.680                       | 5.570        | 5.300     | 5.000       | 8.146       | 8.000         | 4.657       |
| Anus to tip tail | 212                             | -                       | -                           | -            | -         | -           | -            | -             | -            |
| Eggs (L x W)    | 104x41                           | 90x40                    | 115x65                      | -            | -         | -           | 77x33       | -             | 88x38       |
| Host            | Microcavia australis              | Mysateles prehensilis    | Lagidium viscacia           | Dasyprocta leporina | Capromys pilorides | Octodon degus | Abrocoma cinerea | Thrichomys laurentius | Mesomys hispidus |
| Locality        | Argentina                         | Cuba                     | Argentina                   | Trindade     | Cuba      | Argentina    | Andes da Bolivia | Brazil         | Brazil       |
| Author/Year     | Freitas-Texeira *et al.* (1937)  | Vigueras (1943)          | Schuurmans *et al.* (1951)  | Cameron & Reesal (1951) | *Hugot (1986) | Barus (1972) | Quentin *et al.* (1975) | Hugot & Gardner (2000) | Quentin (1969) |
| Anus to tip tail | -                                | -                       | -                           | -            | -         | -           | Present study |

* Measurements in micrometers.
Figure 3. *Helminthoxys freitasi* A) Adult female, general view of the anterior part of the body and alae lateral (thin arrow); B) Cephalic plate, apical view, amphid (asterisk) and two papillae (head arrow); C) Bursal cauda, one pair of papillae (head arrow) and one papillae pedunculate (arrow); D) Detail of the cloaca (C) showing one pair of sessile papillae (large arrow), accessory hook of gubernaculum A); E) Area of the rugosa posterior to the second mamelon; F) Detail of the mamelon surface showing longitudinal striations; G) Anus of the female, ventral view.
Figure 4. Phylogenetic relationships of *Helminthoxys freitasi* isolates from this study and other oxyurids using the MT-CO1. Bayesian 50% majority rule consensus tree after burn-in. Support values are shown at the following nodes, respectively: aLRT, ML-BP, and BPP. Branch lengths are proportional to the mean posterior probabilities of the branch lengths of the sampled trees (scale bar, substitutions per site).
DISCUSSION

The taxonomic characteristics of the genus *Helminthoxys* were the presence of two mamelons in the sub-ventral region of the body, ventral ornamentation, size of the spicule, presence of gubernaculum, and cervical and lateral alae according Quentin, 1973. Morphological characteristics that identify *H. freitasi* are the size of the spicule in male and the position of the vulva in relation to the body length in the female, situated at the posterior part of the body.

In comparison, *H. urichi* is the closest species it resembles based on the proportion of the spicule length to the body length. This characteristic corroborates the evolutionary hypothesis proposed by Hugot (1986), which states that the opening of the posterior vulva in the female body is associated with the elongation of the spicules in males of such species (Hugot, 1986). These two species may be separated by the position and size of the mamelons, in which the males of the species *H. urichi* are further developed.

The genus *Helminthoxys* have a wide distribution within the Neotropical region, extending throughout Central and South America, with each species found associated to a family of host. The species *H. freitasi* was first described infecting the echimyid rodent *Thrichomys laurentius* (Trouessart, 1880). The genus *Thrichomys* is found in open and forested areas in the Caatinga, Cerrado, Chaco, and Pantanal biomes in Brazil, Bolivia, and Paraguay (Patton et al., 2015). The present study reports a new host, the rodent, *Mesomys hispidus* (Desmarest, 1817) 18 also belonging to family Echimyidae, and a new geographical distribution in the Amazon Forest in Brazil. Our findings suggest an association of *H. freitasi* and hosts of the family Echimyidae, enable that this specie could also parasitize others echimyids.

However, the differences in the measurements of morphological structures of the specimens studied compared to the original descriptions may be associated with intraspecific variations relative to adaptive modification to its hosts, and may also be associated with the difference between the body mass of host *Mesomys hispidus* (140–202mm, 160g) and *Thrichomys laurentius* (197–209mm, 267 g) (Patton et al., 2015).

The subfamily Syphaciinae Railliet, 1916 comprises five tribes, including the tribe Protozoophagini composed of three genera, which include the following: *Helminthoxys* Freitas, Lent, and Almeida, 1937; *Wellcomia* Sambon, 1907; and *Protozoophaga* Travassos, 1923, based on morphological characteristics of the genital structures of males and cephalic plaques, according to some synapomorphies that both genera share (Hugot, 1988). Studies based on molecular phylogenies have shown the evolutionary affinity between *Wellcomia* and *Protozoophaga* genera (Nadler et al., 2007). There is, however, no phylogenetic study based on DNA sequences including the genus *Helminthoxys*. In this work, in spite of a limited number of GenBank sequences available, we inferred the phylogenetic relationships of representatives of the tribes Hilgertiini, Passalurini, Syphacinii and Protozoophagiini based on the MT-CO1 gene. The phylogenetic reconstructions obtained by ML and BI revealed *Wellcomia siamensis* as a sister to *H. freitasi*, with high support in all analyses, thus providing support for the tribe Protozoophagiini as a natural group. Our phylogenetic data thus confirms previous works based on morphology (Hugot, 1988; Hugot et al., 2013).

In conclusion, the present study details some morphological characteristics of *H. freitasi* using SEM and light microscopy and contributed with additional taxonomic characters of male and female. This study also contributed the first genetic information for *Helminthoxys*. Our new DNA data suggests a close relationship of *H. freitasi* with the genus *Wellcomia*, corroborating the morphological phylogeny proposed by Hugot (1988). Additionally, *H. freitasi* was recorded for the first time in the Amazon region and parasitizing a new host, *M. hispidus* (Desmarest, 1817) also belonging to family Echimyidae, and a new geographical distribution in the Amazon Forest in Brazil. Our findings suggest an association of *H. freitasi* and hosts of the family Echimyidae, enable that this specie could also parasitize others echimyids.

ACKNOWLEDGMENTS

This study was established under a partnership between the Instituto Federal do Acre (IFAC) and the Instituto Oswaldo Cruz (IOC). We are grateful...
to the DNA-Sequencing Genomic Platform (PDTIS/FIOCRUZ), to the Rudolf Barth Electron Microscopy, and thank Ricardo Baptista Schimidt for the image services, and the Venicio da Costa Ribeiro Junior for the draw enhancement

Instituto de Comunicação e Informação Científica e Tecnológica em Saúde - ICICT/Multimeios. This study received financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior –CAPES and Instituto Oswaldo Cruz (FIOCRUZ). AMI is financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) fellowship grant number 307932/2014-1, and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro), fellow shipgrant number CNE2/2016.

**BIBLIOGRAPHIC REFERENCES**

Abascal, F, Zardoya, R & Telford, M, J. 2010. Translator X: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic acids research, vol. 38, W7-W13.

Anisimova, M & Gascuel, O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Systematic Biology, vol. 55, pp. 539–552.

Barus, V. 1972. Remarks on the Cuban species of the genus Helminthoxys (Nematoda, Syphaciidae). Folia Parasitologica, vol. 19, pp. 105-111.

Bell, KC, Calhoun, KL, Hoberg, E, P, Demboski, JR & Cook, J A. 2016. Temporal and spatial mosaics: Deep host association and geographic drivers shape genetic structure in a widespread pinworm, Rauschineria eutamii (Nematoda: Oxyuridae). Biological Journal of the Linnean Society, vol. 119, pp. 397-413.

Cameron, TWM & Reesal, MR. 1951. Studies on the endoparasitic fauna of Trinidad mammals. VII. Parasites of hystricomorph rodents. Canadian Journal of Zoology, Ottawa, vol. 29, pp. 276-289.

Freitas-Teixeira, JF, Lent H & Almeida, JL. 1937. Pequena contribuição ao estudo da Fauna helminthologica de Argentina (Nematoda). Memórias do Instituto Oswaldo Cruz, vol. 32, pp. 195-209.

Guindon, S, Dufayard, JF, Lefort, V, Anisimova, M, Hordijk, W & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology, vol. 59, pp. 307-21.

Hasegawa, H, Sato & Torii, H. 2012. Redescription of Enterobius (Enterobius) macaci Yen, 1973 (Nematoda: Oxyuridae: Enterobiinae) Based on Material Collected from Wild Japanese Macaque, Macaca fuscata (Primates: Cercopithecidae). Journal Parasitology, vol. 98, pp. 152-159.

Hugot, JP, Feiliu, C & Ribas, A. 2013. Laoxyuris laonasti n. gen., n. sp. (Nematoda: Syphacinae) parasite of Laoastes enigmamus (Rodentia: Diatomyidae): morphology, biology, taxonomy, phylogeny. Infection Genetic Evolution, vol. 13, pp. 213-221.

Hugot, JP & Gardner, SL. 2000. Helminthoxys abrocomae n.sp. (Nematoda: Oxyurida) from Abrocoma cinerea in Bolivia. Systematic Parasitology, vol. 47, pp. 223–230.

Hugot, JP. 1986. Etude morphologique d’Helminthoxys urchi (Oxyurata, Nematoda), parasite de Dasyprocta aguti (Caviomorpha, Rodentia). Bulletin du Muséum National d’Histoire Naturelle, Série 4, vol. 8, pp. 133–138.

Hugot, JP. 1988. Les nematodes Syphacinae parasites de Rongeurs et de Lagomorphes. Taxinomie. Zoogéographie. Évolution. Mémoires du Muséum national d’histoire naturelle Série A Zoologie, vol. 141, p.p 1-153.

Kang, S, Sultana, T, Eom, KS, Park, YC, Soonthornpong, N, Nadler, SA & Park, JK. 2016. The mitochondrial genome sequence of Enterobius vermicularis (Nematoda: Oxyurida) an idiosyncratic gene order and phylogenetic information for chromadore nematodes. Gene, vol. 429, pp. 87-97.

Kearse, M, Moir, R, Wilson, A, Stones-Havas, S, Cheung, M, Sturrock, S, Buxton, S, Cooper, A, Markowitz, S, Duran, C, Thierer, T, Ashton, B, Meintjes, P & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for
the organization and analysis of sequence data. Bio informatics, vol.28, pp. 1647-1649.

Lefort, V, Longueville, JE & Gascuel O. 2017. SMS: Smart Model Selection in PhyML. Molecular Biology Evolution, vol. 34, pp. 2422-2424.

Liu, GH, Li, S, Zou, FC, Wang, CR & Zhu, XQ. 2016. The complete mitochondrial genome of rabbit pinworm Passalurus ambiguus: genome characterization and phylogenetic analysis. Parasitology Research, vol. 115, pp. 423-429.

Maddison, WP & Maddison, DR. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org.

Miller, MA, Pfeiffer W & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop (GCE), New Orleans, LA pp. 1-8.

Nadler, SA, Carreno, RA, Mejía, A, Madrid, H, Ullberg, J, Pagan, C, Houston, R & Hugot, JP. 2007. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. Parasitology, vol. 134, pp. 1421-1442.

Okamoto, M, Urushima, H, Iwasa M, Hasegawa H. 2007. Phylogenetic Relationships of Rodent Pinworms (genus Syphacia) in Japan Inferred from Mitochondrial COI Gene Sequences. Journal of Veterinary Medicine. Science, vol. 69, pp. 545-547.

Park, JK, Sultana, T, Lee, SH, Kang, S, Kim, HK, Min, GS, Eom, KS, Nadler, SA. 2011. Monophyly of clade III nematodes is not supported by phylogenetic analysis of complete mitochondrial genome sequences. BMC Genomics, vol. 12, pp. 392.

Patton, JL, Pardiñas, UFJ, D’Ellia, G. 2015. Mammals of South America, Volume 2: Rodents. 1384 p. Chicago: University of Chicago Press.

Quentin, J, Courtin, C, Fontecilla, LS, Gallardo, J. 1995. Octodontoxys gigantean n. gen., n.sp., Nuevo nematodo Oxyurinae, parasite de un rodear caviomorfo de Chile. Boletín Chileno de Parasitología, vol. 30, pp. 21-25.

Quentin, JC. 1969. Helminthoxys freitasi n. sp., Oxyure parasite d’un Rongeur Echimyidae Du Bresil. Bulletin du Muséum national d’histoire naturelle. Paris, 3eser., n°167, Zool., vol. 112, pp. 1045-1096.

Ramhaut, A, Suchard, MA, Xie, D, Drummond, AJ. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.

Ronquist, F, Teslenko, M, Van Der Mark, P, Ayres, DL, Darling, A, Höhna, S, Larget, B, Liu, L, Suchard, MA, Huelsenbeck, JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, vol. 61, pp. 539-542.

Schuermans-Stekhoven, JH. 1951. Nematodos parasitos de anfibios, parajos y mamiferos de la Republica Argentina. Acta Zoologica. Lilloana, vol. 10, pp. 315-400.

Sheng, L, Cui, P, Fang, SF, Lin, RQ, Zou, FC & Zhu, XQ. 2014. Sequence variability in four mitochondrial genes among rabbit pinworm (Passalurus ambiguus) isolates from different localities in China. Mitochondrial DNA, Early Online, vol. 26, pp. 501-504.

Sikes, RS. 2016. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal Mammal, vol. 97, pp. 663–688.

Souza, JGR, Lopes Torres, EJ, Garcia, JS, Gomes, APN, Rodrigues-Silva, R & Maldonado, JRA. 2017. Light and scanning electron microscopy study of in vitro effects of artesunate in newly excysted metacercariae of Echinostoma paraensei (Trematoda: Digenea). Experimental Parasitology, vol. 174, pp. 10-16.

Stewart, A, Lowe, A, Smales, L, Bajer, A, Bradley, J, Dwuznik, D, Franssen F, Griffith J, Stuart P, Turner, C, Zalesiny, G & Behnke, JM. 2016. Parasitic nematodes of the genus Syphacia Seurat, 1916 infecting Muridae in the British Isles, and the peculiar case of Syphacia frederici. Parasitology, vol. 145, pp. 269-280.

Swofford, DL. 2002. PAUP*: Phylogenetic Analysis using Parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.
analyses of the complete mitochondrial genomes of the two murine pinworms Aspiculuris tetraptera and Syphacia obvelata. Gene, 585, pp. 71-75.

Xia, X & Lemey, P. 2009. Assessing substitution saturation with DAMBE. The phylogenetic handbook: a practical approach to DNA and protein phylogeny, In book: Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny, Edition: Second, Publisher: Cambridge University Press, Editors: Philippe Lemey, Marco Salemi and Anne-Mieke Vandamme, vol. 2, pp. 615-630.

Xia, X & Xie, Z. 2001. DAMBE: software package for data analysis in molecular biology and evolution. Journal of heredity, vol. 92, pp. 371-373.

Xia, X, Zheng, X, Marco, S, Lu C & Yong, W. 2003. An index of substitution saturation and its application. Molecular Phylogenetics and Evolution, vol. 26, pp.1-7.

Received April 30, 2019.
Accepted September 26, 2019.