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A New Species of *Vampirolepis* (Cestoda: Cyclophyllidea: Hymenolepididae) from the Bat *Artibeus lituratus* (Chiroptera: Phyllostomidae) in the Amazon Rainforest, Brazil

Michele Maria dos Santos [1,2], Raquel de Oliveira Simões [3], Paulo Sérgio D'Andrea [2], Rair de Sousa Verde [4], Arnaldo Maldonado Júnior [2], Reina Isabel Argueta Cartagena [5], Daniel Guimarães Ubiali [5] and José Luis Luque [3,*]

1 Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica 23.890-000, Brazil; mms.ioc.fiocruz@gmail.com
2 Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro 21.040-900, Brazil
3 Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica 23.890-000, Brazil
4 Programa de Pós-Graduação em Sanidade e Produção Animal Sustentável da Amazônia, Universidade Federal do Acre, Rio Branco 69.920-900, Brazil
5 Setor de Anatomia Patológica, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica 23.890-000, Brazil
* Correspondence: luqueufrrj@gmail.com

Abstract: The Amazon biome has a great diversity of bat species. In the state of Acre, Brazil, there is an estimated occurrence of 64 bat species with the species of the genus *Artibeus* as one of the most abundant. Despite their abundance and widespread distribution within the biome, the helminth fauna from Amazonian bats is still poorly known. In this way, the objective of this study is to describe a new species of cestode from the genus *Vampirolepis* found in *A. lituratus*, collected at the Parque Estadual do Chandless, a natural preserved area, located in the Purus River Basin, Southwest Amazon, the state of Acre. The new species of *Vampirolepis* is distinguished from the others by the number and size of hooks, testes disposition, size of the cirrus sac, ovaries and internal and external seminal vesicles. Additionally, molecular study showed that this forms a paraphyletic clade with *Vampirolepis elongatus*.

Keywords: cestodes; bats; *Vampirolepis*; Amazon; Acre

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1. Introduction

The Amazon biome covers parts of Brazil, Venezuela, Colombia, Peru, Bolivia, Ecuador, Suriname, Guyana and French Guiana [1]. This biome contains the greatest biodiversity in the world [2]. An estimated 64 bat species occur in the Brazilian state of Acre, with those of the *Artibeus* genus being most common [3]. Despite their abundance and widespread distribution within the biome, the helminth fauna of bats from Acre is still unknown [4,5].

Artibeus lituratus has wide distribution on the Neotropical region, occurring from Mexico to Bolivia and in all of Brazil and northern Argentina, as well as in Trinidad and Tobago and the Lesser Antilles. In Brazil, the species is known for its high abundance. Although *A. lituratus* can be found in conserved environments, it is one of the species better adapted to altered and urban environments. It has a predominantly frugivorous diet, although it can also feed on floral resources, leaves and insects such as beetles [6].

Cestodes of the genus *Vampirolepis* (Cyclophyllidea: Hymenolepididae) are distributed worldwide and known to parasitize only Chiropterans [7–11]. In South America, 14 species belonging to the *Vampirolepis* genus have been reported [4,7,9–21]

Species of the *Vampirolepis* genus have heterogeneous morphological features, such as different testes positions and uterus structures, forming distinct morphological groups. This heterogeneity was demonstrated by phylogenetic studies of mammalian hymenolepidids with armed scolices [22,23].

In this study, we describe a new species of *Vampirolepis* found in the bat *A. lituratus* collected in the Brazilian Amazon rainforest.

2. Materials and Methods

2.1. Animals and Study Area

This study was carried out in Parque Estadual Chandless (PEC), a natural preserved area of 695,303 hectares, located in the Purus River Basin, Southwest Amazon [24]. The PEC vegetation is a mosaic composed mainly of open forest without bamboo, bamboo-dominated open forest and bamboo-dominated palm tree open forest [25]. The bats were captured in three plots measuring 100 m long and 10 m wide. In each plot, we carried out two nights of sampling, totaling six nights from October to November 2019. At each sampling site, we used eight mist nets (12 × 3 m, mesh 19 mm, Ecotone®) installed continuously inside the plots at ground level. The captures began at sunset and ended 6 h after net installation, with inspections every 20 min. The total sampling effort was 288 mist net-hours (mnh), where 1 mnh is equal to a 12 m net opened for 1 h. Captures were performed under a license granted by the competent environmental agency (SISBIO 71451-4), and all procedures followed protocols that were approved by the Animal Use Ethics Committee of Universidade Federal do Acre (License CEUA 28/2019). The captured bats were placed in cotton containment bags for weighing, body measurement and morphological identification according to the keys of [26–28]. Type host specimen was deposited in the Wide Mammals Reservoirs Integrated Collection (COLMASTO) of the Oswaldo Cruz Institute in Rio de Janeiro.

2.2. Parasite Collection

A total of nine specimens of *Artibeus lituratus* were euthanized and immediately examined for the presence of helminths in the gastrointestinal tract and cavities. The cestodes collected from were isolated, washed and relaxed in 0.85% physiological saline solution and then fixed in 70% ethanol for subsequent analysis [29].

2.3. Integrative Taxonomy

2.3.1. Morphological Studies

For light microscopy, 10 specimens were submitted to a regressive process of carmine staining [29]. Drawings for the morphometric features were made using a Nikon Eclipse E200 light microscope with the aid of a camera lucida. Images were captured using a digital camera coupled to a Zeiss Axioscope A1 light microscope with the TCapture version 5.1.1.1 software. The cestodes were identified according to [11,12,16,30]. The measurements are given in millimeters and the means are followed by the ranges. For the structures that each specimen presents more than once, as suckers, proglottids, ovaries, cirrus sacs, external seminal vesicles, internal seminal vesicles, seminal receptacles, testes, eggs, onchospheres and hooks, the values presented are from the general means of the structures measured of all specimens. Type specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) in Rio de Janeiro.

2.3.2. Histological Analyses

Eight specimens were fixed in 10% buffered formalin and routinely processed for histology with staining by hematoxylin and eosin [26]. The slides were evaluated with optical microscopy [31].

2.3.3. Molecular and Phylogenetic Analyses

The genomic DNA was isolated from two specimens. The samples were washed individually in distilled water for 24 h to remove the 70% ethanol. Total DNA was extracted using a QIAmp DNA Mini Kit according to manufacturer's protocol. The partial nuclear small subunit (SSU) ribosomal RNA gene (18S rDNA) sequence was amplified with conventional polymerase chain reaction (PCR) using the primers SSU-A (forward, 5′-AAAGATTAAGCCATGCATG-3′) and SSU-22R (reverse, 5′-GCCTGCTGCCTTCCTTGGA-3′) to produce amplicons (~400 bp) [32].

The PCR was performed with 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM $MgCl^2$, 0.2 mM of each primer, 1.5 U of Platinum Taq polymerase (Invitrogen) and 25–50 ng of genomic DNA in a final volume of 50 µL. Then, the sample was subjected to an initial cycle of 3 min at 94 °C, followed by 40 cycles at 94 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s in a programmable thermal controller (BioRad Thermal Cycler T100). Products were submitted to electrophoresis in 2% agarose gels and visualized using GelRed nucleic acid gel stain (Biotium, Hayward, CA, USA).

The samples were sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the ABI 3730 DNA Analyzer (Genomic Sequencing Platform/FIOCRUZ), according to the manufacturer's instructions. Sequences were edited, aligned and analyzed using pairwise/Blast/NCBI comparisons with the Geneious R9.1 software [33] to provide consensus sequences. The sequence obtained was deposited in the GenBank under accession number SUB13030012.

The online PhyML 3.0 software [34] was used to reconstruct phylogenies based on the maximum likelihood (ML) approach. The model of nucleotide evolution was selected with Smart Model Selection (SMS), run in PhyML [35], using the Bayesian information criterion. Node support was computed with nonparametric bootstrap percentages and 1000 pseudo-replications (ML-BP) [36]. Bayesian phylogenetic inferences (BIs) were carried out using MrBayes version 3.2.6 [37] in XSEDE using the CIPRES Science Gateway [38]. The Bayesian analyses were performed using a single K2 + G model. Markov chain Monte Carlo samplings of each matrix were performed for 10,000,000 generations with four simultaneous chains in two runs. Branch supports in Bayesian trees with Bayesian posterior probabilities (BPP) were assessed from trees that were sampled every 100 generations, after removal of a 25% burn-in fraction. A database was constructed using sequences obtained here plus representative sequences from the species of this family available in the GenBank. The sequence of *Choanotaenia infundibulum* (Cestoda: Dilepididae) was added as outgroup.

3. Results

3.1. Bat Capture

From the nine specimens of *Artibeus lituratus* captured, euthanized and examined for the presence of helminths, five specimens were infected by helminths and of these only one was infected by the new species of *Vampirolepis* recorded here.

3.2. Vampirolepis spp.

The records of *Vampirolepis* spp. in South America with their host(s) species, geographical distribution and data sources are listed in Table 1.

Table 1. List of *Vampirolepis* spp. recorded in South America with their host(s) species, number of rostellar hooks, geographical distribution and data sources.

Species	Number of Rostellar Hooks	Host(s)	Locality	Reference(s)
Vampirolepis artibei (Rutkowska & Zdzitowiecki, 1980)	20–23 (n = 14)	*Glossophaga soricina*	Peru	[4,12,13,17]

Table 1. *Cont.*

Species	Number of Rostellar Hooks	Host(s)	Locality	Reference(s)
Vampirolepis bihamata (Sawada & Harada, 1986)	88–90 (n = 2)	*Micronycteris minuta*	Bolivia	[4,14,17,21]
Vampirolepis christensoni (Macy, 1931)	36–38 (n = 14)	*Nyctinomops laticaudatus*, *Molossidae* gen. sp. and *Chiroptera* gen. sp.	Brazil, Bolivia and Paraguay	[4,12,15–17]
Vampirolepis crassihamata (Sawada & Harada, 1986)	22 (n = 1)	*Molossus molossus*	Bolivia	[4,14,17,21]
Vampirolepis decipiens (Diesing, 1850)	44 (n = 9)	*Cynomops abrasus brachymeles*, *Eumops patagonicus* (as *Eumops bonariensis beckeri*), *Eumops glaucinus*, *E. perotis*, *Molossops temminckii and Pteronotus parnellii rubiginosus* (as *Chilonycteris rubiginosa*)	Brazil, Argentina and Paraguay	[4,9,12,16–18]
Vampirolepis elongatus (Rêgo, 1962)	32 (n = 20)	*Molossus rufus*, *Glossophaga soricina*, *Artibeus obscurus* (as *A. fuliginosus*), *A. planirostris*, *Artibeus lituratus*, *Pygoderma bilabiatum*, *Phyllostomus hastatus*, *Platyrrhinus helleri* and *Chiroptera* gen. sp.	Brazil, Argentina, Paraguay, Bolivia and Peru	[4,7,12,16,17,19]
Vampirolepis guarany (Rêgo, 1961)	24–26 (n = 13)	*Molossus crassicaudatus*, *Molossus* sp. and *Chiroptera* sp.	Paraguay and Brazil	[9,16,17,20]
Vampirolepis longisccata (Sawada & Harada, 1986)	36–38 (n = 2)	*Molossus molossus*	Bolivia	[14,17,21]
Vampirolepis temmincki (Vaucher, 1986)	29–34 (n = 13)	*Molossus temminckii*	Paraguay	[4,9,17]
Vampirolepis promopsis (Vaucher, 1986)	45 (n = 1)	*Promops centralis*	Paraguay	[4,9,17]
Vampirolepis phyllostomi (Vaucher, 1982)	42–52 (n = 8)	*Phyllostomus hastatus* and *Eumops bonariensis beckeri*	Peru, Bolivia and Paraguay	[4,9,14,17]
Vampirolepis santacruzensis (Sawada & Harada, 1986)	23 (n = 1)	*Molossus molossus*	Bolivia	[4,14,17,21]
Vampirolepis mazanensis (Vaucher, 1986)	37–40 (n = 4)	*Saccopteryx bilineata* and *Rhynchonycteris* naso	Peru	[10,17]
Vampirolepis pandoensis (Sawada & Harada, 1986)	41 (n = 1)	*Eptesicus furinalis*	Bolivia	[4,14,17,21]

Legend: (n) = Number of specimens analyzed.

3.3. *Vampirolepis dalvae n. sp.*

Description

Comparative structural measurements of *Vampirolepis dalvae* n. sp. and *Vampirolepis* spp. from the Neotropical region with 20 to 30 rostellar hooks are presented in Table 2.

Table 2. Comparative structural measurements of *Vampirolepis dalvae* n. sp. and *Vampirolepis* species registered in the Neotropical region, presenting from 20 to 30 rostellar hooks. The measurements of *Vampirolepis dalvae* n. sp. are given as means followed by the range. The number of specimens measured and the number of structures measured are shown in parentheses. All the measurements are given in millimeters.

Structural Measurements (mm)	***Vampirolepis dalvae* n. sp.**	***Vampirolepis artibei* (Rutkowska & Zdzitowiecki, 1980)**	***Vampirolepis crassihamata* (Harada & Sawada, 1986)**	***Vampirolepis guarany* (Rêgo, 1962)**	***Vampirolepis santacruzensis* (Harada & Sawada, 1986)**
Length	40.81 (n = 1)	40 (n = 1)	32 × 0.5 (#) (n = 1)	30.15−36.83 (n = 13)	65 × 1.2 (#) (n = 1)
Neck (L × W)	-	-	-	-	0.9 × 0.4 (n = 1)
Scolex (L × W)	0.363 × 0.342 (0.236–0.47 × 0.281–0.4) (n = 10)	0.211 × 0.276 (n = 14)	0.560 × 0.476 (n = 1)	0.415 × 0.581 (n = 13)	0.420 × 0.476 (n = 1)
Rostellar sac (L × W)	0.134 × 0.099 (0.097–0.155 × 0.069–0.123) (n = 5)	(0.096–0.125) (W) (n = 14)	0.245 (*) (n = 1)	0.363 × 0.231 (n = 13)	0.315 × 0.345 (n = 1)
Rostellum (L × W)	0.069 × 0.073 (0.057–0.089 × 0.052–0.111) (n = 10)	(0.074–0.089 × 0.077–0.083) (n = 14)	0.189 × 0.161 (n = 1)	0.209 × 0.182 (n = 13)	0.231 × 0.175 (n = 1)
Suckers (L × W)	0.108 × 0.106 (0.078–0.144 × 0.086–0.128) (n = 10; ns = 36)	(0.089–0.104) (*) (n = 14)	(0.133–0.140 × 0.154) (n = 1)	0.126 (*) (n = 13)	(0.147–0.161 × 0.126–0.161) (n = 1)
Mature proglottids (L × W)	0.086 × 0.340 (0.061–0.105 × 0.247–0.413) (n = 1; ns = 12)	(0.089–0.126 × 0.4–0.43) (n = 1)	-	0.174 × 2.53 (n = 13)	-
Gravid proglottids (L × W)	0.178 × 0.476 (0.135–0.267 × 0.333–0.547) (n = 2; ns = 8)	(0.148–0.193 × 0.34–0.445) (n = 1)	-	-	-
Testes (L × W)	0.02 × 0.021 (0.018–0.023 × 0.018–0.026) (n = 1; ns = 11)	(0.042–0.053 × 0.055–0.072) (n = 1)	(0.084–0.091 × 0.067–0.084) (n = 1)	0.191 × 0.104 (n = 13)	(0.105–0.119 × 0.070–0.084) (n = 1)
Testes arrangement	Transversal row	Transversal row	Transversal row	Transversal row	Transversal row
Cirrus sac (L × W)	0.084 × 0.028 (0.077–0.095 × 0.027–0.030) (n = 1; ns = 3)	(0.096–0.103 × 0.029–0.048) (n = 1)	(0.140–0.175 × 0.028) (n = 1)	0.182 × 0.043 (n = 13)	(0.154–0.175 × 0.035) (n = 1)
Cirrus	0.032 (*) (n = 1; ns = 1)	0.024 (L)/0.06 (*) (n = 1)	-	-	-
External seminal vesicle (L × W)	0.027 × 0.025 (0.018–0.034 × 0.018–0.031) (n = 1; ns = 4)	(0.054–0.072 × 0.030–0.038) (n = 1)	(0.091–0.098 × 0.028) (n = 1)		(0.070–0.084 × 0.028–0.053) (n = 1)
Internal seminal vesicle (L × W)	0.027 × 0.030 (0.018–0.039 × 0.026–0.034) (n = 1; ns = 4)	-	0.070–0.105 × 0.028 (n = 1)	-	(0.070–0.084 × 0.028–0.053) (n = 1)
Seminal receptacle (L × W)	0.019 × 0.026 (0.015–0.028 × 0.018–0.042) (n = 1; ns = 4)	(0.082–0.144 × 0.048–0.108) (n = 1)	0.063–0.077 × 0.035 (n = 1)	0.174 × 0.052 (n = 13)	(0.105–0.154 × 0.084) (n = 1)
Ovary (L × W)	0.039 × 0.087 (0.036–0.039 × 0.076–0.1) (n = 1; ns = 4)	(0.054–0.067 × 0.156–0.175) (n = 1)	-	0.783 (n = 13)	-
Vitelline gland (L × W)	-	(0.040–0.050 × 0.060–0.075) (n = 1)	(0.049 × 0.021–0.028) (n = 1)	0.348 (n = 13)	(0.140 × 0.077–0.084) (n = 1)
Eggs (L × W)	0.055 × 0.046 0.045–0.066 × 0.042–0.053 (n = 1; ns = 10)	(0.035–0.045) (*) (n = 1)	-	0.034 (n = 13)	(0.046–0.056 × 0.035–0.046) (n = 1)
Onchosphere	0.037 × 0.030 (0.030–0.050 × 0.025–0.038) (n = 1; ns = 11)	(0.025–0.029 × 0.022–0.027) (n = 1)	-	-	(0.028–0.039 × 0.025–0.028) (n = 1)
Onchosphere hooks (L)	-	0.016 (n = 1)	-	0.015 (n = 13)	0.014 (n = 1)
Hooks (N)	(21–25) (n = 10)	(20–23) (n = 14)	22 (n = 1)	(24–26) (n = 13)	23 (n = 1)

Table 2. *Cont.*

Structural Measurements (mm)	*Vampirolepis dalvae* n. sp.	*Vampirolepis artibei* (Rutkowska & Zdzitowiecki, 1980)	*Vampirolepis crassihamata* (Harada & Sawada, 1986)	*Vampirolepis guarany* (Rêgo, 1962)	*Vampirolepis santacruzensis* (Harada & Sawada, 1986)
Hook blades (L × W)	-	-	-	(0.014–0.017) (n = 13)	-
Hooks (L)	0.020 (0.016–0.030) (n = 10; ns = 43)	(0.019–0.020) (n = 14)	0.053 (n = 1)	0.05 (n = 13)	0.046 (n = 1)

Legend: (mm) = millimeters; (#) = Strobile; (L) = Length; (W) = Width; (*) = Diameter; (n) = Number of specimens measured; (ns) = Number of structures measured; (-) = No information; (N) = Number of hooks.

The scolex has four unarmed rounded suckers (Figures 1A,B and 2A) and a rostellum armed with a single circular row of 25 fraternoid hooks (Figures 1B,C and 2A) inserted in the rostellar sac (Figures 1A,B and 2A). The number of hooks was confirmed in all five specimens that had the rostellum analyzed in greater detail. Furthermore, the rostellar hooks have blades slightly longer than the guards (Figures 1D and 2B). Proglottids craspedote, numerous mature proglottids (Figure 2C) more wide than long, and while the gravid ones (Figure 3A) gradually become more long than wide and contain oval oncospheres (Figure 3A,B).

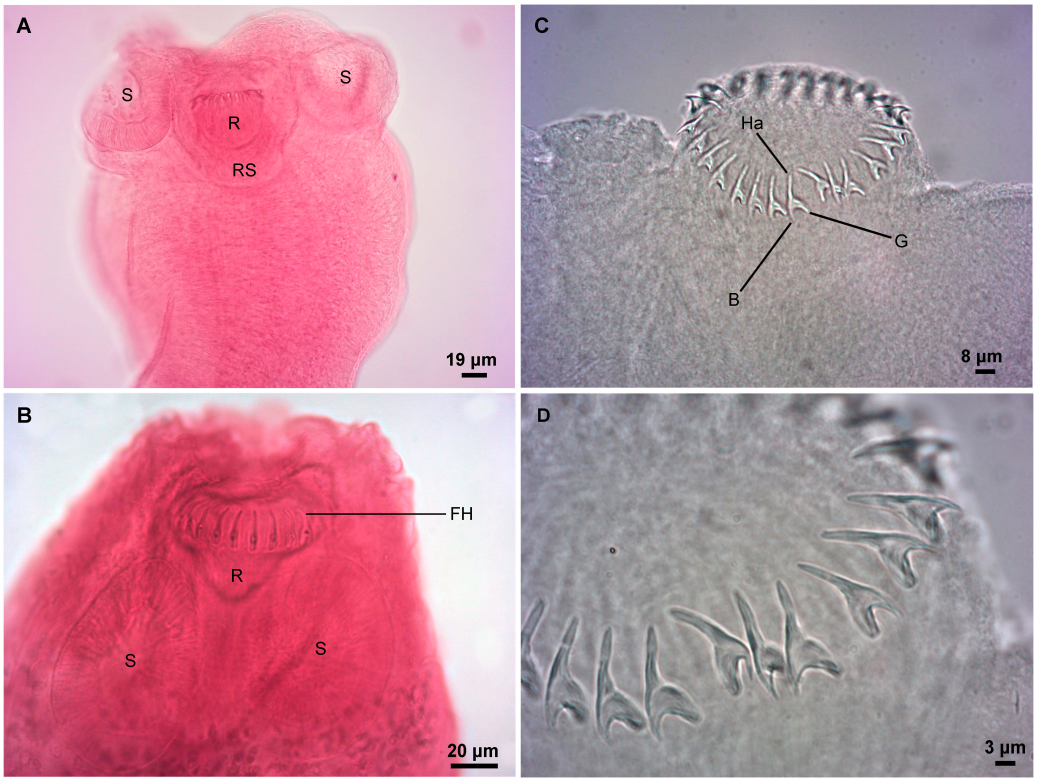

Figure 1. Light microscopy of *Vampirolepis dalvae* n. sp. structures. (**A**) Scolex with unarmed suckers (S) and a rostellum (R) inserted in a rostellar sac (RS); (**B**) scolex with unarmed suckers (S) and a rostellum (R) armed with fraternoid hooks (FH); (**C**) rostellar hooks with each hook formed by a handle (Ha), a guard (G) and a blade (B); (**D**) detail of fraternoid hooks with the blades slightly longer than the guards.

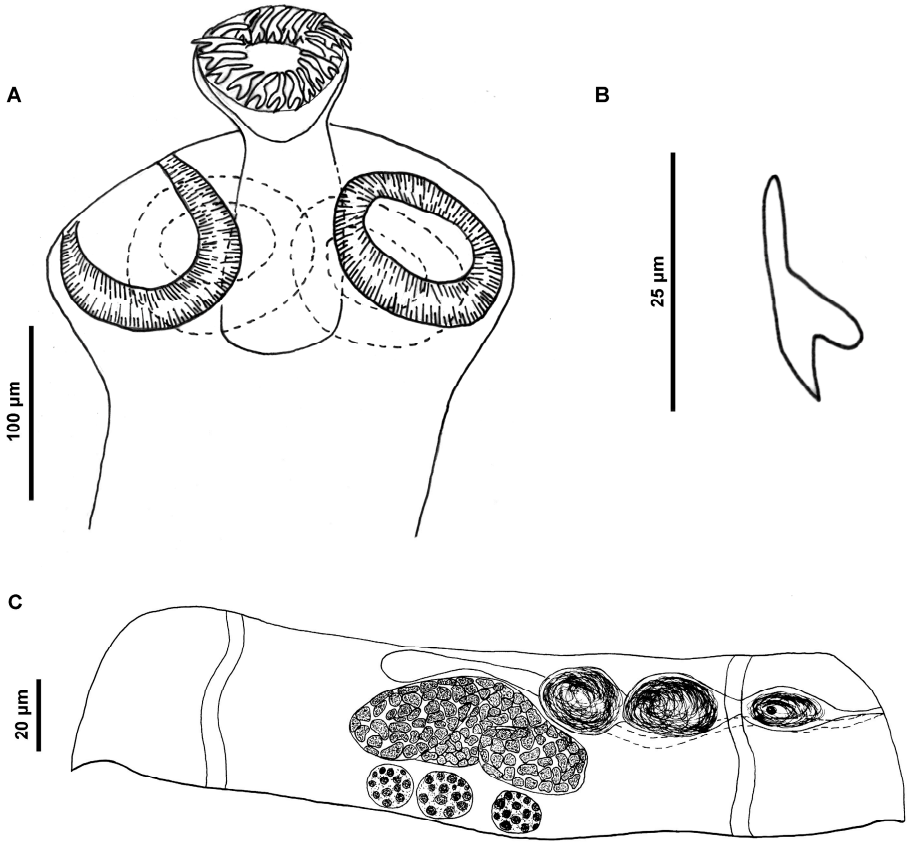

Figure 2. Light microscopy drawing of *Vampirolepis dalvae* n. sp. structures. (**A**) Escolex structure. (**B**) Rostellar hook with the blade being slightly longer than the guard. (**C**) Mature proglottid with dextral genital atrium aperture near the middle of the margin, followed by the external cirrus sac, that reaches the longitudinal excretory canals, the internal seminal vesicle and the external seminal vesicle. Vagina opens in the genital atrium, passes beneath the cirrus sac and then enlarges to form the oval seminal receptacle. Moreover, an irregularly lobate ovary is present at the anterior part of the proglottid. In the posterior region of the proglottid, there are three testes arranged in a transverse row.

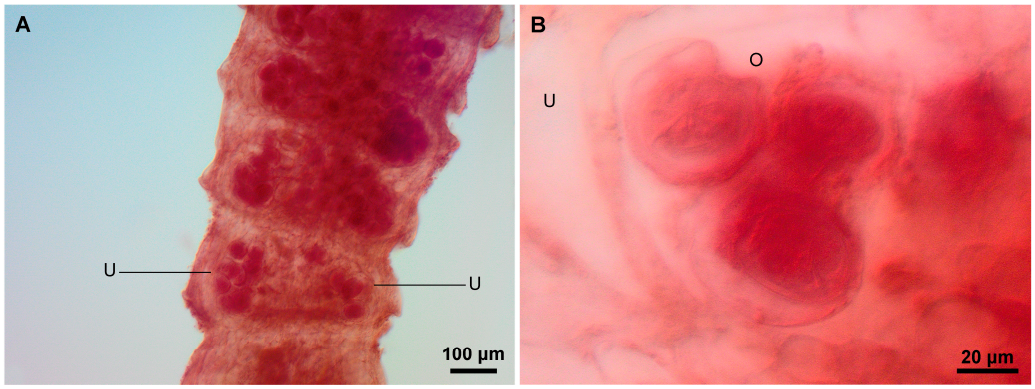

Figure 3. Light microscopy of *Vampirolepis dalvae* n. sp. structures. (**A**) Gravid proglottids with a sac-like bilobed uterus (U) filled with oncospheres; (**B**) detail of the oncospheres (O) in the uterus (U).

Single sets of reproductive organs, with a unilateral and dextral genital atrium aperture near the middle of proglottid margins, are shown in Figures 2C and 4A. The presence of three oval testes, two situated on the antiporal side and one on the poral side, is shown in Figures 2C and 4B. The external cirrus sac reaches the longitudinal excretory canals. Internal seminal vesicle enlarges, connecting to the proximal portion of the cirrus sac. The external

seminal vesicle directly dorsal to the seminal receptacle in the anterior half of the proglottid is shown in Figures 2C and 4A,D. An ovary irregularly lobate and transversely elongated in the anterior half of the proglottid is shown in Figures 2A and 3C. The vagina opens in the genital atrium, passes beneath the cirrus sac, extends medially beyond the longitudinal excretory canals and then enlarges to form the oval seminal receptacle (Figure 2C). The vitelline gland is compact and located posteriorly to the ovary at the posterior region of the mature proglottid.

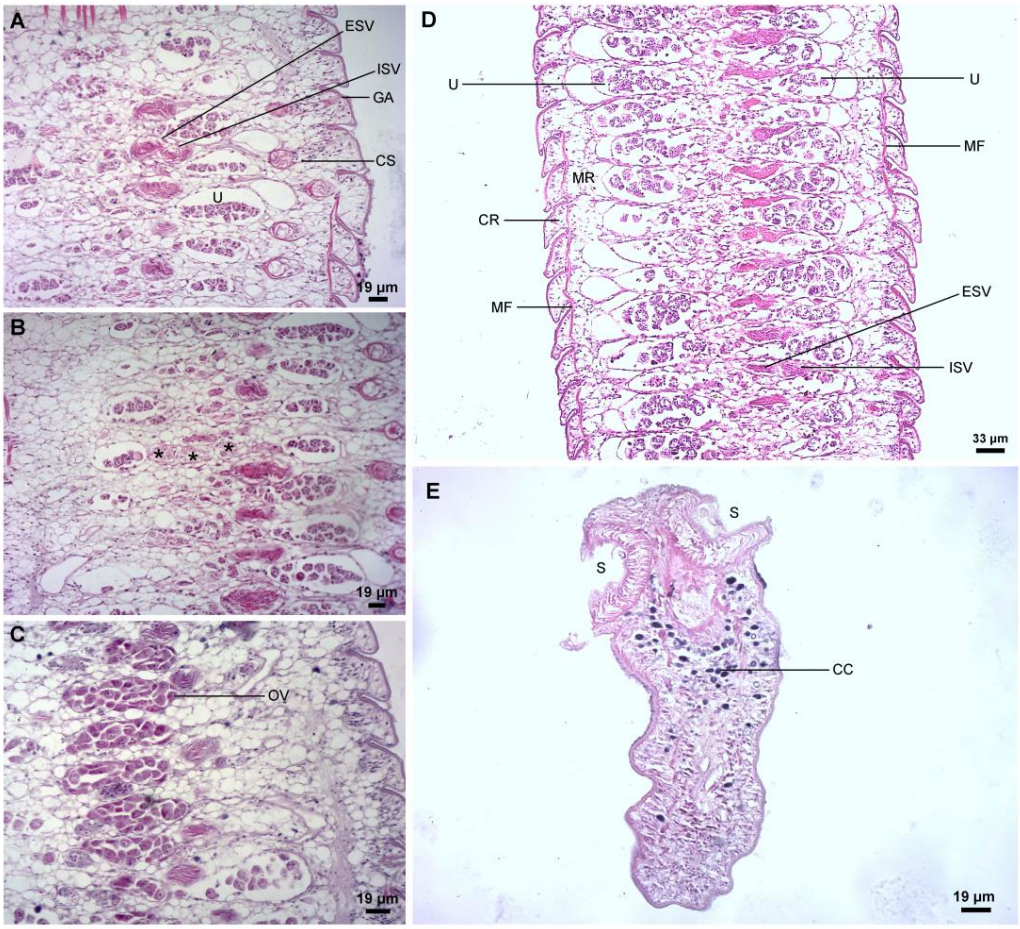

Figure 4. Histological sections of *Vampirolepis dalvae* n. sp. structures. (**A**) Mature proglottid with the genital atrium (GA) aperture near the middle of proglottid margin, one lobe of uterus (U) and the cirrus sac (CS), followed by the internal seminal vesicle (ISV) and the external seminal vesicle (ESV); (**B**) mature proglottid with three testes (*) arranged in a transverse row; (**C**) mature proglottid with irregularly lobate ovary (OV); (**D**) detail of mature proglottids with sac-like bilobed uterus (U), internal seminal vesicle (ISV), external seminal vesicle (ESV) and muscular fibers (MF) separating the cortical region (CR) from the medullary region (MR); (**E**) detail of the muscular suckers (S) and the calcareous corpuscles (CC) scattered in the parenchyma.

The histological sections of the mature proglottids allowed for observing testes forming a transverse row in the posterior field (Figure 4B), internal and external seminal vesicles connected to the cirrus sac by a narrow duct (with these three structures full of sperm) and the dextral genital atrium aperture situated near the middle of the proglottid margin (Figure 4A). Moreover, there is a sac-like bilobed uterus that gradually enlarges until filling the entire gravid proglottid (Figure 4D). In the scolex region, suckers present radial striations of the muscular fibers and many calcareous corpuscles scattered in the parenchyma (Figure 4E). Additionally, muscles within the parenchyma separate the cortical from the medullary region (Figure 4D).

Taxonomic summary

Type host: *Artibeus lituratus* (Olfers, 1818); specimen deposited in the COLMASTO, LBCE-21906.

Type locality: Parque Estadual do Chandless–Manoel Urbano Municipality, the state of Acre, Brazil.

Site of infection: Small intestine.

Prevalence: 11.1% (1 infected of 9 examined).

Mean intensity: 440 (440 helminths collected from one infected host).

Mean abundance: 48.8 (440 helminths collected from nine hosts captured).

Etymology: The new species is named in honor of Dalvanete Maria dos Santos, mother of the first author, affectionately called Dalva.

Specimens deposited: 01 holotype CHIOC-39993 and 02 paratypes CHIOC-39629

This article was registered in the Official Register of Zoological Nomenclature (ZooBank) as: urn:lsid:zoobank.org:act:5ECCF588-5899-4AA1-A01D-33B48431B452.

3.4. Molecular and Phylogenetic Analyses

The 18S rDNA gene matrix had 11 taxa and 519 characters, of which 312 were constant and 57 were parsimony-informative variables. The Bayesian analyses returned a mean estimated marginal likelihood of −1437.9073, with a median value of −1437.569. ESSs for all parameters were above 100 effectively independent samples and much larger for most parameters, demonstrating the robustness of our sampling.

Tree topologies produced with different optimality criteria (ML and BI) were similar, with little variation in node support values (Figure 5). *Vampirolepis dalvae* n. sp. formed a clade with *Vampirolepis elongatus* and *Hymenolepis nana* (ML-BP = 100%; Bayesian posterior probabilities (BPP) = 99). Species belonging to the family Hymenolepididae formed a polytomy.

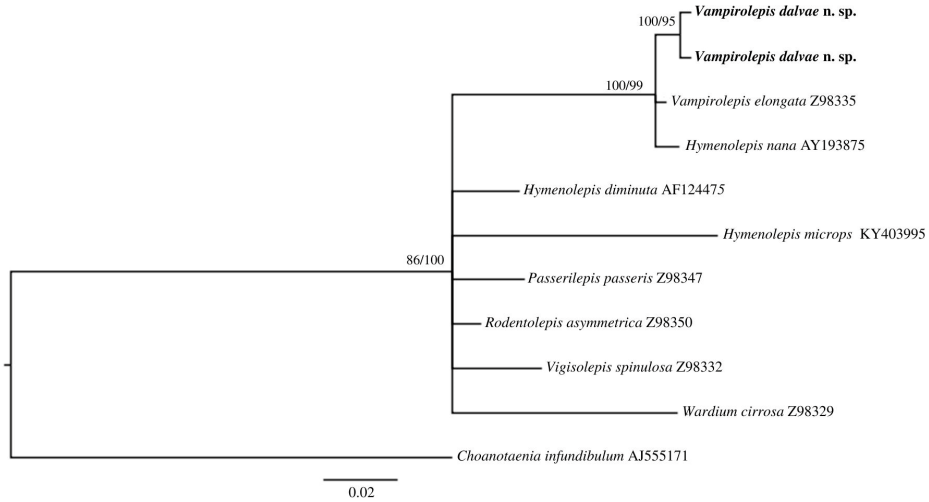

Figure 5. Bayesian analysis tree of 18S rRNA gene sequences inferring the phylogenetic relationship between *Vampirolepis dalvae* n. sp. (in bold) and hymenolepidid sequences from the GenBank. The node values are for the maximum likelihood (ML) and Bayesian inference (BI) methods, left to right.

4. Discussion

Hymenolepididae is a large family that includes genera that mainly parasitize birds and mammals [39]. Among mammals, most genera and species occur in Soricomorpha, Chiroptera and Rodentia [39–42].

Spassky erected *Vampirolepis* to include hymenolepidid species that parasitize bats [43]. Previously, these species were described as belonging to *Hymenolepis*, parasitizing only bats and characterized by a rostellum armed with about 50 fraternoid-type hooks and testes arranged in a straight line. The species included by this author were first found in bats, but

later several species were also found in shrews and one in birds [11]. Currently, this genus contains 78 species parasitizing bats [11,15,17,21] and another 15 infecting shrews [21].

The *Vampirolepis* genus was reviewed and some classification criteria for this complex group were established [11]. Among the criteria are all species parasitize bats and the presence of an armed rostellum including from 18 to more than 50 fraternoid-type hooks with long and thin handles. The blades are short and the guards are thick, with the guard being as long as the blade or longer. The uterus has two wings, initially with labyrinthic or reticulate walls, then growing to a two-winged sac until occupying the whole proglottid, between the excretory ducts. Moreover, the cirrus sac is pyriform with a well-developed internal vesicle, reaching and sometimes slightly extending beyond the level of the excretory ducts, with the cirrus being smooth or with tiny spines, and eggs characterized as oval, sometimes with a thick external envelope [11].

The description of almost all species of *Vampirolepis* was based only on morphological characteristics, not using genetic identification as an additional tool, making it difficult to carry out a more assertive long-term study of the relationships between the species and the family [9,10,12,14,17,23,44]. Even after the revision proposed by Vaucher [11], the genus remains large [44].

The genera *Rodentolepis* and *Potorolepis* were also erected, as *Vampirolepis*, to clarify this complex issue within *Hymenolepis*. The first one includes species that parasitize rodents and the second includes species found parasitizing marsupials [43,45,46].

Furthermore, the genus *Sawadalepis*, which includes the only species *Sawadalepis prima*, was erected with some morphological similarities to *Vampirolepis* recorded. Among these similarities observed by these authors are the hook morphology, testes arrangement and embryophore structure in the egg. However, other characteristics, such as the position of the dorsal osmoregulatory canals in relation to ventral canals, the sinistral arrangement of the genital pores and the vagina structured into two parts, the sacciform uterus (with ventral and dorsal diverticula, extending bilaterally beyond longitudinal osmoregulatory canals when fully developed) and spherical eggs (with a thick outer coat), differed from *Vampirolepis* [44]. Thus, the morphological features of the new species presented here are not included in *Sawadalepis* criteria, but are similar to the *Vampirolepis* criteria proposed by Vaucher [11], such as the shape of rostellar hooks and morphology of mature and gravid proglottids.

In addition, the new species differs from the 14 species known from the Neotropics by the number of rostellar hooks. Only *V. artibei*, *V. crassihamata*, *V. guarany* and *V. santacruzensis* present from 20 to 30 hooks, close to the number of the new species (25 hooks). *Vampirolepis dalvae* n. sp. differs from *V. artibei* by its larger scolex. In contrast, the new species is characterized by the smallest rostellum and by presenting a smaller rostellar sac than *V. crassihamata*, *V. guarany* and *V. santacruzensis*.

With regard to the reproductive organs, the new species presents testes arranged in a straight line, perpendicular to the longitudinal axis of the proglottid, as in *V. artibei*, *V. crassihamata*, *V. guarany* and *V. santacruzensis*. However, it can be distinguished from them by the following smaller structures: the testes, cirrus sac and internal and external seminal vesicles. The seminal receptacle is the smallest one. In addition, *Vampirolepis dalvae* n. sp. has a larger ovary than *V. artibei* and a smaller one than *V. guarany*. Unfortunately, this latter structure's measurements for *V. crassihamata* and *V. santacruzensis* were not given by the authors, making comparison impossible.

An analysis with light microscopy of histological sections can help to elucidate structures that cannot be seen clearly with light microscopy of the regressive process of staining using carmines. When analyzing such histological sections, it is possible to identify some characteristic of reproductive organs. In *Vampirolepis dalvae* n. sp., it was possible to observe numerous calcareous bodies in the scolex region and muscular suckers with radial striations of the muscular fibers. Furthermore, muscular fibers in the parenchyma of the proglottids separating the medullary region from the cortical region were observed. These characteristics are determinants of the Cyclophyllidians [26]. Even though this technique

helps through a complementary diagnosis, histological sections were essential to elucidate the reproductive structures and their positions, adding knowledge about hymenolepidid features.

The present species is grouped together with *V. elongatus*, the only species of the genus with available sequences deposited in the GenBank. The phylogenetic analyses confirm that the new species belongs to *Vampirolepis*. Species of the Hymenolepididae family show polytomy, demonstrating that the relationship of this group is not well defined. Furthermore, more molecular data on this family are necessary to clarify the phylogenetic relationship. In fact, a non-monophyletic group formed with mammalian hymenolepidid taxa using a Bayesian analysis of the partial sequence of the 28S rDNA gene was observed [47]. These authors also suggested sequencing samples of cestodes from rodents, shrews and bats to adequately understand the relationship between the parasite and host. Thus, data on hymenolepidids such as molecular sequences and phylogenetic analyses from mammalian hosts are still scarce, limiting the understanding of the morphology, biology and phylogeny of this taxon. The present study contributes to future studies of hymenolepidids' phylogenetic position and evolutionary patterns, especially for *Vampirolepis*.

5. Conclusions

The use of integrative taxonomic tools is essential to elucidate the complexity of the hymenolepidid group. The morphological features of *Vampirolepis dalvae* n. sp. are in accordance with the classification criteria proposed by Vaucher [11]. Among these features, the presence of an armed rostellum and number of rostellar hooks, as well as testes arrangements and other characteristics of the reproductive systems, were more relevant. The phylogenetic analyses showed polytomy among species of Hymenolepididae, demonstrating that the relationship of this group is not well defined.

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Data Availability Statement: The data are available upon reasonable request to the corresponding author.

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