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A paruterinid metacestode in the liver of a Neotropical bat (*Molossus molossus*)

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ABSTRACT

We describe a case of microscopic liver lesion caused by larval cestode in a frugivorous bat (*Molossus molossus*) from the Western Brazilian Amazon. Histopathological analysis of liver indicated the occurrence of metacestode associated with multifocal histiocytic response and the phylogenetic analysis of Cox-I and 18S rDNA genes indicated that the parasite belonged to the family Paruterinidae. This is the first identification of cestodes of the family Paruterinidae in bats and may suggest a broad range of paruterinid tapeworm hosts to be investigated in the Brazilian Amazon.

1. Introduction

The family Paruterinidae Fuhrmann 1907 (Cestoda: Cyclophyllidea) includes a heterogeneous and polyphyletic group of cyclophyllideans that does not have taxonomic characteristics compatible with other families of cestodes; its main morphological characteristic is the paruterine organ [1]. There is no consensus on the taxonomic classification of this family. Georgiev and Kornyushin [1] considered paruterinids as a single family but also presented an alternative taxonomic treatment of this cestode group as consisting of several distinct family-group taxa: the family Paruterinidae (*Paruterina, Cladotaenia, Culcitella, Laterotaenia,* and *Matabelea*), the family Biuterinidae Meggitt, 1927 (*Biuterina, Parvirostrum, Dyctiterina, Spasskyterina, Sphaeruterina, Neyraia, Triaenorhina, Notopentorchis, Troguterina, Francobona,* and *Orthoskarlabinia*); the family Anonchotaeniidae Matevosyan, 1965 (*Anonchotaenia, Mogheia*); the subfamily Rhabdometrinae Matevosyan, 1965 (*Rhabdometra, Metroliasthes, Lyruterina, Ascometra,* and *Octopetalum*).

Birds are the main definitive hosts of paruterinid tapeworms; however, data on the biological cycle and intermediate hosts of many species within the family remain unknown [1]. In the Palaearctic region, the adult parasites of *Dictyterina cholodkowskii* were described in shrikes [2], while the genus *Biuterina* occurs in passeriforms of the families Alaudidae and Emberizidae [3]. In Africa, the genus *Biuterina* has been described in passeriforms and coraciiforms [4]. The genus *Paruterina* has been described in owls in North America [1,5], and the genus *Anonchotaenia* has been described in passeriforms in both North [6] and South America [7]. Hawks and eagles are the main definitive hosts of the genus *Cladotaenia* in North America [8–10], Europe [11–13], Asia and Africa [1], while metacestode forms are described in rodents, such as *Clethrionomys gapperi*, *Napaeozapus insignis*, *Peromyscus maniculatus*, *Tamias* spp., *Apodemus microps*, *Microtus pennsylvanicus*, and *Ondatra zibethica*, in which cestode larvae are predominantly located in the liver [10,14–16].

Rodents (*Mus muculus* and *Peromycus maniculatus*), experimentally infected by oral route with the eggs of *Cladotaenia globifera* obtained from a hawk (*Accipiter striatus velox*) presented hepatic lesions caused by metacestodes from 10 to 575 days post-infection [10]. Macroscopically, parasitic lesions exhibited cysticercus characteristics, with a well-defined cyst wall and translucent to opaque appearance and were observed mainly in the peripheral areas of the liver [10].

Intestinal cestodes of the families Anoplocephalidae (*Cycloskrjabinia* sp., *Mathevotaenia* spp. and *Atriotaenia hastati*) and Hymenolepididae (*Hymenolepis* spp. and *Vampirolepis* spp.) have been described in Neotropical bats [17], but until recently, only hymenolepidids *Vampirolepis elongatus* [18] and Hymenolepididae gen. sp. were described in Amazonian bats [19]. Therefore, this is the first characterization of histopathology and molecular identification of a metacestode detected in the liver of a Neotropical bat (*Molossus molossus*) from the Brazilian

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Western Amazon region.

2. Case report

The liver samples used in the present study were collected during fieldwork to evaluate health status of Neotropical bats at Pauini City, Amazonas State, located in the Western Brazilian Amazon (7.7292° S, 68.3339° W), during August 2005. The study was approved by the Ethics Committee on the Use of Animals of Evandro Chagas Institute under number 010/2009.

Twenty-two bats belonging to the families Molossidae (5 *Molossus molossus*) and Phyllostomidae (4 *Artibeus* sp.; 1 *Artibeus lituratus;* 1 *Carollia brevicauda;* 3 *Carollia perspicillata;* 1 *Glossophaga soricina;* 4 *Macrophyllum macrophyllum*; 1 *Sturnira lilium;* 1 *Vampyressa pusilla;* and 1 *Vampyrodes caraccioli*) were captured, euthanized and submitted to gross evaluation and organ sample collection. From each bat, one hepatic fragment was fixed in 10% formalin solution for histopathology, and another hepatic fragment was first stored in liquid nitrogen in the field and subsequently frozen at −20 °C for molecular analysis.

Liver samples fixed in formalin solution were embedded in paraffin, cut at 5 μm thick and stained with hematoxylin-eosin (HE). The samples were examined under a light microscope (Eclipse Ni−U, Nikon).

No gross liver lesions were observed in any of the 22 bats. In the insectivorous bat, *Molossus molossus* (1/22; 4.5%), histopathology revealed two foci of parasitic larva covered by tegument that presented a solid parenchymatous body and a developing invaginated scolex, unarmed rostellum (in a process of formation) and developing suckers, with morphologic characteristics compatible with the class Cestoda (Fig. 1) [20]. The cestode larvae also did not present evident primary lacuna, cercomer and/or vesicles, and based on these morphological characteristics, the metacestode was classified as merocercoid type according to the larval cestodes classification proposed by Chervy [21]. Here, the metacestodes were provisionally designated *M. molossus* hepatic metacestodes.

The parasitic larvae were surrounded by a moderate number of xanthomized histiocytes, few lymphocytes and rare eosinophils. Compressed and vacuolated hepatocytes surrounding the larvae foci were also observed. In one focus, fibrosis within inflammatory response was evident in the Picrosirius stain (Fig. 1).

Total DNA of the hepatic tissue was extracted with DNeasy Blood and Tissue kit (QIAGEN), according to manufacturer's instructions. Total DNA from the liver sample was eluted in a 200-μL final volume and subsequently stored at −70 °C until further molecular characterization of the parasite. The total DNA of the hepatic sample was submitted to molecular tests by two PCR protocols for the partial amplification of the Cox-I [22] and 18S rDNA [23] genes of cestodes. Positive (DNA of *Echinococcus vogeli* metacestode-positive sample) and negative (Nuclease-free water) controls were included in all assays, and samples that presented PCR products with 446 bp (Cox−I) and 450 bp (18S rRNA) in the automated detection by capillary electrophoresis (QIAxcel DNA Screening Kit, QIAGEN) were considered positive.

PCR products were purified with ExoSAP-IT® (GE Healthcare) and sequenced in the forward and reverse directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the BigDyeX Terminator® Purification Kit (Applied Biosystems) in an AB3500 (Applied Biosystems) automatic sequencer. All reactions were performed according to the manufacturer's instructions.

Cox-I (GenBank accession number MG865289) and 18S (GenBank accession number MG860906) consensus sequences were obtained with the Geneious 8.1.3 software and subsequently submitted to the BLASTn (Basic Local Alignment Search Tool) platform (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The results of both the Cox-I and 18S sequences showed similarity to sequences of the family Paruterinidae.

To evaluate the phylogenetic relationship of *M. molossus* hepatic metacestode with cestode families, Cox-I consensus of the parasite was aligned with nucleotide sequences representing 8 families of the order

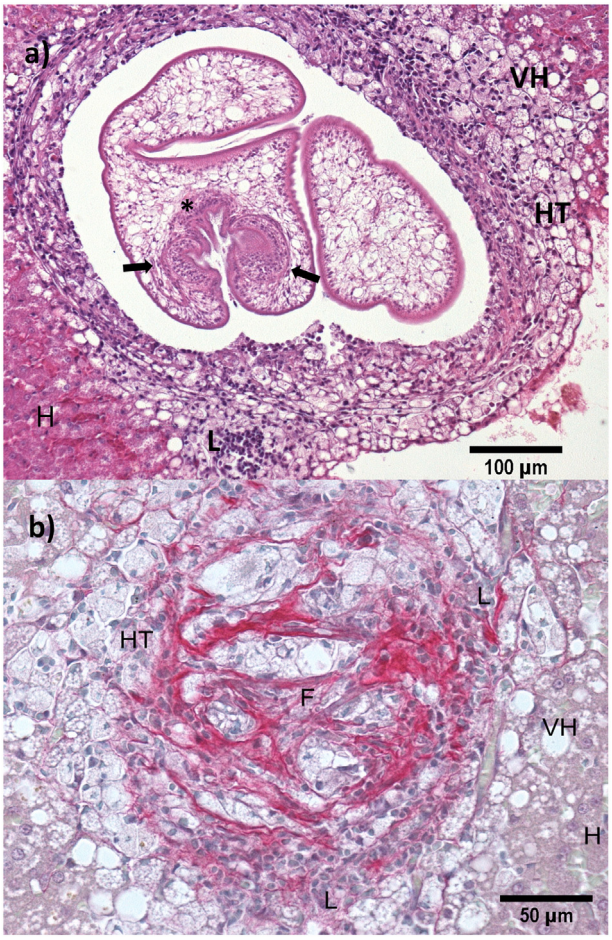

Fig. 1. Histopathological sections of *M. molossus* hepatic metacestode. **a)** Metacestode surrounded by xanthomized histiocytes. Hematoxylin-eosin, 20×. **b)** Inflammatory process with histiocytes associated with metacestode (not presented in this cut section). Note: fibrosis is in red. Picrosirius stain, 40×. Legend: Large arrows, suckers; *, developing rostellum; H, hepatocytes; L, lymphocytes; HT, histiocytes; VH, vacuolated hepatocytes; and F, fibrosis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cyclophyllidea, and the 18S consensus was aligned with representative sequences of the families Taeniidae and Paruterinidae. Phylogenetic analyses were inferred by maximum-likelihood using MEGA v.7.0 software, and the analysis of Cox-I (319 bp) was developed under the GTR + G nucleotide substitution model and 18S region (285 bp) using the HKY + G model. Phylogenetic trees obtained by the analysis of both Cox-I and 18S (Fig. 2) confirmed that the sequences of the *M. molossus* hepatic metacestode clustered next to the sequences of the family Paruterinidae.

The identity matrix indicated that *M. molossus* hepatic metacestode presented a percentage of nucleotide identity with the family Paruterinidae ranging from 82.3–87.2% in Cox-I (Supplementary table 1) and 90.6–95.4% in 18S (Supplementary table 2). The partial sequence of Cox-I presented a greater percentage of nucleotide identity with the species *Dictyterina cholodkowskii* (GenBank accession number KF685925) obtained from a Chinese shrike (*Lanius tephronotus*), and 18S presented higher identity with a sequence of *Cladotaenia* sp. (GenBank accession number KP241938) described in an eagle (*Aquila nipalensis*) from China.

3. Discussion

Small mammals, including Neotropical bats, may act as hosts of a

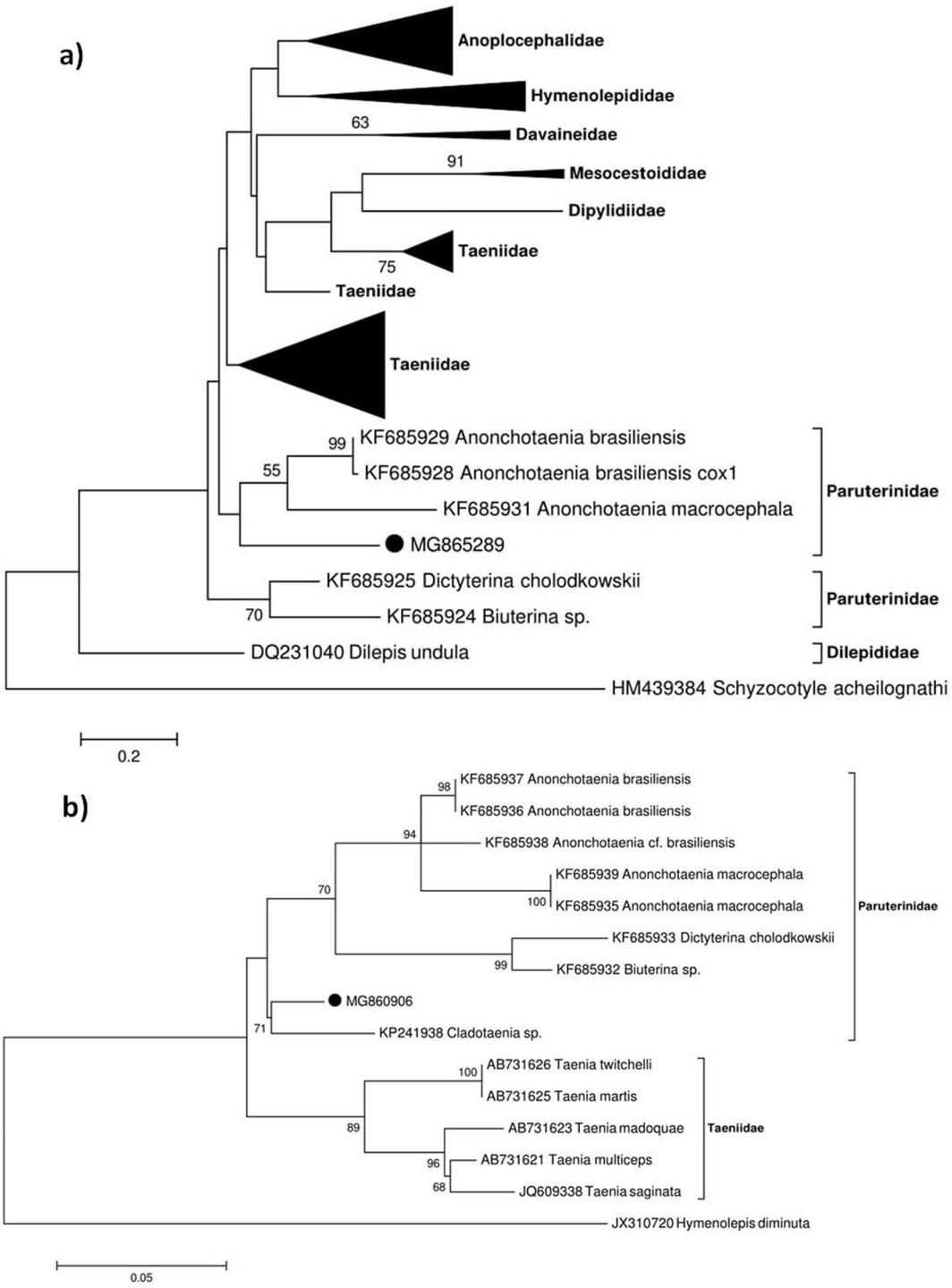

Fig. 2. Maximum-likelihood trees based on partial nucleotide sequences: **a)** Cox-I (319 bp) gene of 99 cyclophyllideans. A sequence of *Schyzocotyle acheilognathi* was used as the outgroup; **b)** The 18S (285 bp) gene of 15 cyclophyllideans. A sequence of *Hymenolepis diminuta* was used as the outgroup. Sequences are identified by GenBank accession number, followed by species identification. The sequences obtained from *M. molossus* hepatic metacestode are highlighted (●). Bootstrap values (1000 replicates) > 60% are listed at the nodes. Scale bars indicate nucleotide substitutions per site.

great variety of cestode species, but despite parasites of the families Anoplocephalidae, Davaineidae and Hymenolepididae having been previously described in Old World and New World bats, the life cycles of these chiropterans cestodes are unknown [24]. Thus, the present description of the occurrence of a metacestode in the liver of a Neotropical bat may also indicate new perspectives on the host variety and ecological distribution of cestode parasites among bats from the Amazon.

Cestodes of the family Paruterinidae may occur as adult worm in rare or accidental forms in amphibians, waterfowl and mammals [1], and although there is no report of infection in humans, the hypothesis of accidental infection in humans has recently been considered by paruterinid tapeworms from the feces of birds [25].

The genus *Cladotaenia* exhibits non-specificity of intermediate hosts [10], and both rodents and insectivore mammals can be intermediate hosts for this genus [1]. Similar to other intermediate hosts of the

family Paruterinidae, Neotropical bats of the species *M. molossus* (Chiroptera, Molossidae) have an exclusive insectivorous diet and are widely distributed in the Brazilian territory [26]. Interestingly, the evidence of this bat species in the natural cycle of the *M. molossus* hepatic metacestode has been considered since owls may be definitive hosts for some paruterinids [1,5] and Molossidae bats are prey for owls in tropical regions [27–31].

In intermediate hosts, paruterinid metacestodes (also known as merocercoids or cladothyridia) have mainly been described in the hepatic tissue of several rodent species in viable forms, as here described in the liver of bats, or as dead/disintegrating forms [5,10]. The intensity of the inflammatory response surrounding metacestodes may be directly proportional to the parasite viability and host-parasite relationship [32–34]. Here, the *M. molossus* hepatic metacestode was observed in a viable form within lesion, despite the occurrence of a surrounding moderate inflammatory reaction.

Eberhard [20] indicates that the morphological characteristics observed by histological analysis are important for metacestodes diagnosis, but these analyses have limitations for species classification. Therefore, the morphological characteristics observed in *M. molossus* hepatic metacestodes were used for the classification as merocercoid larval type according to the parameters proposed by Chervy [21].

The *M. molossus* hepatic metacestode presented incomplete development, invaginated scolex, developing suckers, and lack of primary lacuna, which are morphological characteristics similar to those of the metacestodes of the genus *Cladotaenia* [10]. Based on these characteristics, the metacestodes found in the liver of bats were morphologically compatible with the merocercoid type observed in the family Paruterinidae [21].

We did not observe the presence of hooks in the *M. molossus* hepatic metacestode but could not confirm whether this feature is a specific characteristic of the parasite or whether the absence of hooks was related to the stage of larval development, as Freeman [10] indicated the occurrence of hooks in more advanced stages of the development of *Cladotaenia* metacestodes.

We used a molecular approach to better characterize the taxonomy of the parasite, and the results of the phylogenetic analysis of Cox-I and 18S genes indicated that the *M. molossus* hepatic metacestode belongs to family Paruterinidae and grouped within a sister clade of the family Taeniidae. Thus, the present results corroborated recent molecular studies that indicated that the family Paruterinidae represents a polyphyletic group with a sister group relation with the family Taeniidae [35–38]. The precise phylogenetic relationships of the family Paruterinidae are not yet completely understood, as only a small number of nucleotide sequences in this family are currently available [36,39]. Further research may help to clarify the taxonomy and the natural cycle of the *M. molossus* hepatic metacestode.

4. Conclusion

The histological and molecular results provide strong evidence that the cestode larva found in the bat liver belongs to the family Paruterinidae. To our knowledge, this report is the first study of hepatic lesions associated with a metacestode and the first record of a paruterinid metacestode in chiropterans. These results indicate the need for additional ecological, parasitological and molecular studies to evaluate the geographic and host distribution of paruterinid tapeworms in the Amazon.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2019.01.007.

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