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Eimeria spp. (Apicomplexa: Eimeriidae) of marsupials (Mammalia: Didelphimorphia) in southern Bahia, Brazil

Eimeria spp. (Apicomplexa: Eimeriidae) de marsupiais (Mammalia: Didelphimorphia) na região sul da Bahia, Brasil

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Abstract

The occurrence of *Eimeria* Schneider, 1875 in mammals of the order Didelphimorphia indicates the infection-predisposition of these animals, which in turn is mainly determined for their eating habits. The objective of this work was to evaluate the parasitism of *Eimeria* spp. in marsupials of the Atlantic Forest of the southern region of Bahia. Fecal samples were collected from marsupials captured in the regions of Ilhéus, Una, Belmonte and Mascote, with traps of the Sherman model (23 × 8 × 9 cm), Tomahawk (50 × 17 × 17 cm) and pitfall and analyzed by Sheather's modified centrifugal-flotation method. Oocysts were identified by microscopical evaluation of their morphology and morphometry. *Didelphis aurita* Wied-Neuwied, 1826, *Gracilinanus agilis* Burmeister, 1854, *Monodelphis americana* Müller, 1776, *Marmosa demerarae* O. Thomas, 1905 and *Marmosa murina* Linnaeus, 1758 were parasitized by *Eimeria philanderi* Lainson & Shaw, 1989 and *Eimeria gambai* Carini, 1938. Mixed parasitism for these two coccidia was observed in two of the 56 marsupials sampled. In conclusion, this work registers new hosts for *E. philanderi* and *E. gambai*, as well as the state of Bahia as a new distribution site for these coccidia.

Keywords: Atlantic forest, parasitism, Eimeria philanderi, Eimeria gambai.

Resumo

A ocorrência de *Eimeria* Schneider, 1875 em mamíferos da ordem Didelphimorphia, indica a predisposição à infecção desses animais, que, por sua vez, é determinada principalmente por seus hábitos alimentares. O objetivo do presente trabalho foi avaliar o parasitismo por *Eimeria* spp. em marsupiais da Mata Atlântica da região Sul da Bahia. Amostras fecais foram coletadas de marsupiais capturados nas regiões de Ilhéus, Una, Belmonte e Mascote, com armadilhas do modelo de Sherman (23 × 8 × 9 cm), Tomahawk (50 × 17 × 17 cm) e queda e analisado pelo método de centrífugo flutuação modificado de Sheather. Os oocistos foram identificados pela avaliação microscópica de sua morfologia e morfometria. *Didelphis aurita* Wied-Neuwied, 1826, *Gracilinanus agilis* Burmeister, 1854, *Monodelphis americana* Müller, 1776, *Marmosa demerarae* O. Thomas, 1905 e *Marmosa murina* Linnaeus, 1758 foram parasitados por *Eimeria philanderi* Lainson & Shaw, 1989 e *Eimeria gambai* Carini, 1938. Parasitismo misto para esses dois coccidios foi observado em dois dos 56 marsupiais amostrados. Em conclusão, este trabalho registra novos hospedeiros para *E. philanderi* e *E. gambai*, bem como o estado da Bahia como um novo local de distribuição para esses coccidios.

Palavras-chave: Mata atlântica, parasitismo, Eimeria philanderi, Eimeria gambai.

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Introduction

Eimeria Schneider, 1875 is an intracellular protozoan that infects a wide range of vertebrate hosts. Transmission occurs by ingestion of sporulated oocysts, by direct fecal/oral contact, or by ingestion of contaminated food or water (URQUHART et al., 1987; ZANETTE et al., 2008). Infection by this protozoan can be asymptomatic or cause mild to moderate disease in domestic and wild animals (ZANETTE et al., 2008).

Eimeria species have already been described in Didelphis aurita (Wied-Neuwied, 1826) (CARINI, 1936, 1938; TEIXEIRA et al., 2007), Caluromys philander Linnaeus, 1758 (CARINI, 1937), Didelphis virginiana Kerr, 1792 (JOSEPH, 1974), Philander opossum Linnaeus, 1758 and Caluromys philander Linnaeus, 1758 (LAINSON & SHAW, 1989), Marmosops noctivagus Tschudi, 1844 and Marmosa constantiae O. Thomas, 1904 (HECKSCHER et al., 1999), Myrmecophaga tridactyla Linnaeus, 1758 (FREITAS et al., 2006) and Didelphis albiventris Lund, 1840 (ZANETTE et al., 2008), Didelphis marsupialis Linnaeus, 1758 (VALERIO-CAMPOS et al., 2015) and Gracilinanus agilis Burmeister, 1854 (STRONA et al., 2015); however, some of these species possibly have been

erroneously identified, or at least they were not clearly described and differentiated. Furthermore, based on the scientific literature, there are no reports of *Eimeria* spp. from marsupials in the southern region of Bahia, Brazil, as well as are totally unknown their geographical distribution and host specificity. With this in mind, we designed the current study to survey coproparasites and identify *Eimeria* spp. from fecal samples of marsupials captured in forest areas of the Atlantic Forest of the Southern Bahia region.

Material and Methods

This study covered 14 forest areas in four municipalities in the southern region of the State of Bahia, with three areas of cocoa agroforestry (cabruca) located in the rural area of Ilhéus (areas one to three), and eleven forest areas located in the municipalities of Una, Mascote and Belmonte (areas four to 14) (Figure 1). The study region has a tropical climate and tropical forest vegetation predominates (SAMBUICHI, 2002). All areas were located between 42 m and 100 m above sea level and were georeferenced with the Global Positioning System (GPS).

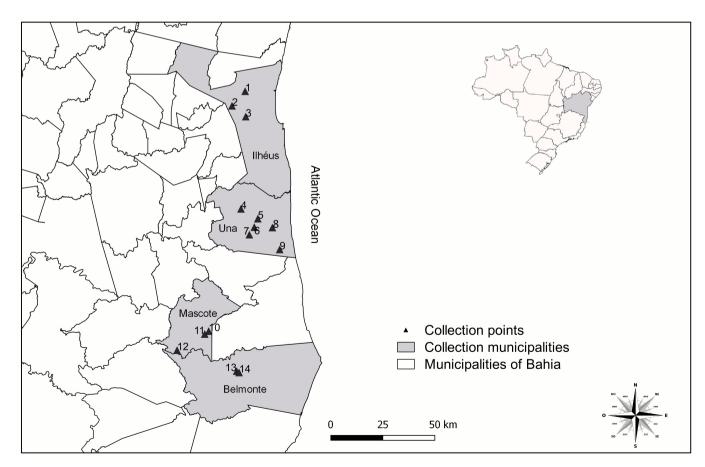


Figure 1. Map with data and areas of capture and collection of fecal samples of marsupials in southern Bahia, Brazil. Geographical coordinates of marsupial's collection points: 01: 14°38'15.8"S 39°12'02.3"W; 02: 14°42'11.2"S 39°15'34.8"W; 03: 14°45'04.0"S 39°11'51.2"W; 04: 15°09'57.8"S 39°13'10.1"W; 05: 15°12'35.9"S 39°08'37.4"W; 06: 15°14'53.1"S 39°09'34.3"W; 07: 15°16'54.5"S 39°10'54.2"W; 08: 15°14'59.0"S 39°04'41.0"W; 09: 15°20'53.0"S 39°02'43.5"W; 10: 15°42'53.6"S 39°21'52.6"W; 11: 15°43'40.9"S 39°22'56.7"W; 12: 15°48'01.9"S 39°30'23.8"W; 13: 15°53'40.4"S 39°14'19.2"W; 14: 15°54'03.0"S 39°13'40.4"W.

Marsupial capture was from June 2015 to December 2016 using Sherman ($23 \times 8 \times 9$ cm), Tomahawk ($50 \times 17 \times 17$ cm), and pitfall traps. Each area was divided into three plots, totaling 24 traps per plot and 72 traps per area. The study was licensed by the System of Authorization and Information on Biodiversity (SISBIO) number 17131-4 of the Brazilian Institute of Environment and Natural Renewable Resources (IBAMA) and the Council of Ethics in the Use of Animals of the State University of Santa Cruz - CEUA - UESC (No. 003/2013 and 021/2014).

After identification and collection of fecal samples, euthanasia was performed carried out through the intramuscular administration in the gluteal region of ketamine hydrochloride (30 mg/kg) associated with xylazine hydrochloride (2 mg/kg) according to Cubas et al. (2007). Euthanized marsupials were housed at the Universidade Estadual de Santa Cruz - Mammal Collection (CMARF-UESC). Feces with an approximate amount of 180-200 mg were collected and stored in 1.5 mL centrifuge tubes containing 2.5% potassium dichromate (K2Cr2O7) in a ratio of 1: 3 and transported to the Laboratory of Veterinary Parasitology of the Universidade Estadual de Santa Cruz (LAPVET-UESC).

The samples were processed and examined by the modified flotation technique with 1,43 (g/L) density sucrose solution (500 g sucrose, 350 ml water) by centrifugation (5 min at 2000 rpm) described by Sheather (1923) and modified by Duszynski & Wilber (1997). All samples were observed under an Olympus BX51 microscope on the 20× objective and confirmed with a 40× objective. Positive samples were kept at 20–24°C, allowing aeration for seven days to ensure complete oocyst sporulation. Sporulated oocysts were observed, photomicrographed and measured using a 100× objective microscope and immersion oil, coupled to a digital camera connected to a computer using the Image - Pro Express 6.0 program. For specific identification, morphological

and morphometric guidelines described by Duszynski & Wilber (1997) and Berto et al. (2014) were used.

Results

Fifty-six samples from *Didelphis aurita*, *Gracilinanus agilis*, *Monodelphis americana*, *Marmosa demerarae* and *Marmosa murina* were analyzed, and 58.92% (33/56) were positive for *Eimeria* oocysts (Table 1). After sporulation and morphological and morphometric study of oocysts and sporocysts (Table 2), were identified *Eimeria philanderi* Lainson and Shaw, 1989 (Figure 2A, B) and/or *Eimeria gambai* Carini, 1938 (Figure 2C, D). Mixed parasitism was observed in *M. murina* and *M. demerarae*.

Eimeria philanderi (Figure 2A, B) was identified from *D. aurita*, *M. americana*, *M. demerarae* and *M. murina*, at Una, Belmonte and Mascote in Southern Bahia, Brazil (Figure 1). Their oocysts sporulated in 7-8 days and were observed as sub-spherical, with a double-layered wall (the inner one being dark and occupying about 1/4 of the wall, and the outer one brown, with striations and projections occupying around 3/4 of the wall). Micropyle and oocyst residue were absent, but a polar granule was present. The sporocysts were ovoid, with prominent Stieda bodies, although the sub-Stieda was barely discernible. Parastieda bodies were absent. Sporocyst residue was present in the form of a few dispersed granules. The accompanying sporozoites had two refractile bodies. The morphometries of *E. philanderi* isolated from each host species are compared in Table 2.

Eimeria gambai (Figure 2C, D) was identified from G. agilis, M. demerarae and M. murina, at Una, Belmonte and Mascote in Southern Bahia, Brazil (Figure 1). Their oocysts sporulated in 7-8 days and were observed as ellipsoids, with a double-layered

Table 1. Distribution of *Eimeria* species related to marsupials and their respective collection points as indicated in Figure 1, in southern Bahia, Brazil.

Hosts	Eimeria spp.	No of samples/Positive	Locality	
Didelphis aurita	Eimeria philanderi	06/02	Area 7, Area 8	
Monodelphis americana	Eimeria philanderi	07/04	Area 4, Area 14	
Gracilinanus agilis	Eimeria gambai	05/04	Area 12, Area 14	
Marmosa demerarae	Eimeria philanderi	09/05	Area 4, Area 7, Area 8	
	Eimeria gambai		Area 4, Area 7, Area 8	
Marmosa murina	Eimeria philanderi	29/18	Area 4, Area 6, Area 7, Area 8, Area 9, Area 10, Area 11, Area 13, Area 14	
	Eimeria gambai		Area 8, Area 10, Area 12	

Table 2. Morphometry of oocysts and sporocysts of *Eimeria* spp. identification of fecal samples of marsupials, from southern Bahia, Brazil.

	species	Oocyst		sporocyst	
Hosts		Size (µm)	Morphometric index (μm)	Size (µm)	Morphometric index (μm)
Didelphis aurita	Eimeria philanderi	$23.40 \pm 1.42 \times 22.8 \pm 1.93$	1.02 ± 0.07	$10.76 \pm 1.08 \times 8.65 \pm 0.51$	1.23 ± 0.09
Monodelphis americana	E. philanderi	$20.27 \pm 1.79 \times 19.45 \pm 1.6$	1.04 ± 0.03	$8.74 \pm 2.06 \times 7.81 \pm 1.30$	1.11 ± 0.14
Gracilinanus agilis	Eimeria gambai	25.67 ± 3.22 × 18.67 ± 1.11	1.37 ± 0.16	$9.46 \pm 1.28 \times 8.0 \pm 0.64$	1.18 ± 0.15
Marmosa demerarae	E. philanderi	22.12 ± 1.97 × 19.71 ± 1.51	1.02 ± 0.11	$9.81 \pm 1.94 \times 7.94 \pm 0.83$	1.22 ± 0.16
	E. gambai	$26.30 \pm 2.48 \times 19.54 \pm 1.04$	1.34 ± 0.12	$9.85 \pm 1.52 \times 8.27 \pm 1.12$	1.20 ± 0.21
Marmosa murina	E. philanderi	$23.71 \pm 2.80 \times 20.67 \pm 2.91$	1.15 ± 0.11	$9.86 \pm 1.59 \times 8.06 \pm 1.04$	1.22 ± 0.15
	E. gambai	$24.11 \pm 1.81 \times 18.67 \pm 0.87$	1.29 ± 0.08	$9.61 \pm 1.37 \times 7.48 \pm 0.53$	1.28 ± 0.17

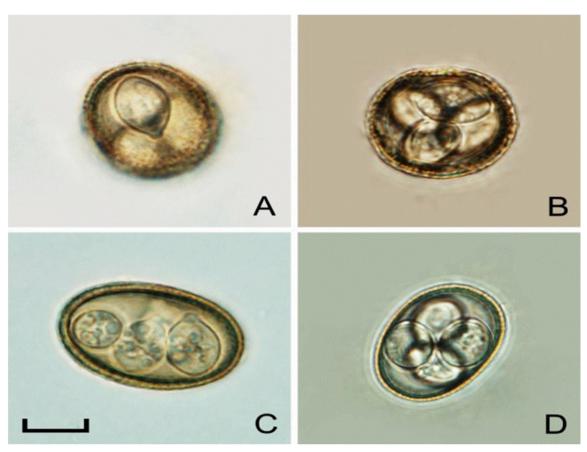


Figure 2. Photomicrographs of recovered and identified oocysts of fecal samples from marsupials in the southern region of Bahia: *Eimeria philanderi* in *Marmosa demerarae* (A) and *Didelphis aurita* (B); and *Eimeria gambai* in *Gracilinanus agilis* (C) and *Marmosa murina* (D) (scale bar = 10μm).

wall (the inner one being dark and occupying about 1/3 of the wall and the outer one brown, with striations and projectoins occupying about 2/3 of the wall). Micropyle, polar granule and oocyst residue were absent. The sporocysts were ovoid, with discrete Stieda bodies and sub-Stieda. Parastieda bodies were absent. Sporocyst residue was present in the form of a few dispersed granules. The accompanying sporozoites had two refractile bodies. The morphometries of *E. gambai* isolated from each host species are compared in Table 2.

Discussion

To the best of our knowledge, the marsupials captured in this study have not been reported previously as hosts for *E. philanderi* and *E. gambai*. This represents the first novel finding of the current report. The oocysts of both species had morphological and morphometric characteristics that were very similar to the original descriptions, and to subsequent follow-up studies (CARINI, 1938; LAINSON & SHAW, 1989; TEIXEIRA et al., 2007).

Eimeriaphilanderi was identified in four species of marsupials in the present study. Their oocysts had morphometries (Table 2) similar to those originally described by Lainson & Shaw (1989). The morphology also corroborates with the studies of Lainson & Shaw (1989) and Heckscher et al. (1999), although in the present study, the presence of sub-Stieda and some differences in

the intensity of roughness, striations, and wall projections were observed.

Eimeria gambai had only previously been isolated from Didelphis aurita (CARINI, 1938; TEIXEIRA et al., 2007). Our current study now extends the host range to G. agilis, M. demerarae and M. murina. The E. gambai oocysts had morphometry (Table 2) similar to that originally described by Carini (1938) and later by Teixeira et al. (2007). The presence of sub-Stieda in the sporocyst, the absence of polar granules, and differences in the intensity of roughness in the wall were observed in oocysts of this study. However, these differences were insufficient to warrant the announcement of a new species.

The delimitation of coccidian species has been discussed since the beginning of parasitology. Traditionally, the description of the new species follow the guidelines of Duszynski & Wilber (1997), who consider that the oocysts should be compared with coccidian species that are feature-similar and belong to the same host family.

Currently with the advent of genetic sequencing techniques, the 18S and 28S rRNA genes and the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene have been recognized as useful loci to complementation of species characterization and in phylogenetic studies (YANG et al., 2015; SILVA-CARVALHO et al., 2018). However, this molecular tool has still limited ability to delimit species because there is no consensus on which genes are primordial and which percentage of genotypic difference separates the species

(SILVA et al., 2016). Anyway, none of these *Eimeria* spp. from marsupials have ever been sequenced in these loci.

In this sense, we endorse Kunz (2002), who states that the criterion for identification of new species can not be just on the basis of a certain number of base exchanges within DNA sequence; therefore, we intend in this work to identify the coccidian species evaluating all the morphological, biological and ecological (host specificity) aspects.

It is also worth mentioning that this paper shows the inconclusive and imprecise taxonomy of marsupial coccidia, since our study prioritized the identification of the species primarily described (CARINI, 1938; LAINSON & SHAW, 1989). This was to the detriment of more recently described species, which were not clearly differentiated from the predecessors (HECKSCHER et al., 1999; TEIXEIRA et al., 2007). In this sense, we emphasize the difficulty in delimiting *Eimeria* spp. from marsupials and correctly assigning them to new or already described species. Such difficulty is associated to the paucity of studies concerning *Eimeria* taxonomy, especially regarding some hosts, such as marsupials.

Conclusion

To the best of our knowledge, this is the first identification of *Eimeria* infection in marsupials captured in the State of Bahia. Although *E. philanderi* and *E. gambai* have already been described in marsupials, there are no records in the scientific literature reporting these two species parasitizing the marsupials *D. aurita*, *G. agilis*, *M. americana*, *M. demerarae*, and *M. murina*. Therefore, this paper has identified new hosts for *E. philanderi* and *E. gambai*, and indicates that the state of Bahia is a new distribution site for these coccidia.

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