A new species of *Eimeria* Schneider, 1875 from the Serra dos Órgãos National Park, Rio de Janeiro, Brazil, with notes on its endogenous development in the montane grass mouse, *Akodon montensis* Thomas, 1913 (Rodentia: Sigmodontinae)

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Abstract
A total of 53 specimens of the montane grass mouse, *Akodon montensis* Thomas, 1913 were collected in the Serra dos Órgãos National Park (SONP) in November 2014 and July 2015. The fecal material was analyzed, and a prevalence of 7.5% was recorded for a new coccidian species of the genus *Eimeria* Schneider, 1875, with part of its endogenous development recorded in the small intestine. The oocysts of a new coccidian species of genus *Eimeria* are ellipsoidal to subspherical. The wall is bi-layered, c. 1.5 μm (1.3–1.6 μm) thick, outer layer rough. Oocyst (*n* = 126) mean length is 25.3 μm (21.0–28.0 μm), with a width of 20.2 μm (17.0–22.0 μm) and mean length/width (L:W) ratio of 1.3 (1.2–1.4). Polar granule is present, with the oocyst residuum as a large spherical to subspherical globule. Sporocyst shape (*n* = 126) is ellipsoidal, with a mean length of 11.8 μm (9.3–14.4 μm), width of 7.9 μm (6.7–9.3 μm), and mean L:W ratio of 1.5 (1.4–1.7). Sporocysts with nipple-like Stieda body and sub-Stieda body are absent. A sporocyst residuum formed by several globules, usually along the sporocyst wall. This is the first record of *Eimeria* in the montane grass mouse from Brazil.

Keywords Rodents · Coccidia · *Akodon montensis* · Atlantic Forest · Histopathology · Cricetidae

Introduction

*Eimeria* spp. are protozoan parasites with worldwide distribution that might cause enteritis in many vertebrate species (Duszynski and Upton 2001). In Brazil, only three species are known to infect Sigmodontinae rodents: *Eimeria oryzomyi* Carini, 1937 from specimens of Oryzomyini rodents; *Eimeria zygodontomyis* Lainson and Shaw, 1990 in *Zygodontomys lasiurus* (= *Necromys lasiurus* (Lund, 1840)), and *Besnoitia akodoni* Dubey, Sreekumar, Rosenthal, Lindsay, Grisard and Vitor, 2003 in *Akodon montensis*.

The rodent family Cricetidae has 130 genera encompassing 681 species, 55 of which are endemic in Brazil. The cricetid genus *Akodon* Meyen, 1833 has 44 species distributed throughout South America (Musser and Carlenton 2005), with 10 species being found in Brazil, on the east coast between Paraíba and Rio Grande do Sul, and inland in highland areas of Minas Gerais, and in the southwest of the state of Mato Grosso do Sul (Bonacciño et al. 2008). The ecology and taxonomy of some common *Akodon* species are well known. They are found in a range of different environments, both pristine and impacted, in addition to their epidemiological importance, as reservoirs of zoonoses (Oliveira et al. 2012; Müller et al. 2013; Teixeira et al. 2014). Four species are...
found in the Brazilian state of Rio de Janeiro—Akodon cursor (Winger, 1887), Akodon serrensis (Thomas, 1902), Akodon paranaensis Christoff et al., 2000, and Akodon montensis Thomas, 1913 (Paglia et al. 2012). These rodents are terrestrial insectivores-omnivores (Graipel et al. 2003).

The present study describes a new species of Eimeria, with notes on its endogenous development in the montane grass mouse, Akodon montensis.

Material and methods

Study area

The study area encompasses a continuous tract of well-preserved Brazilian Atlantic Forest, isolated from human settlements or urban zones. The rodents were captured in the Serra dos Órgãos National Park (SONP), in the municipality of Petrópolis, Rio de Janeiro State, Brazil (22° 27′ 49″ S, 43° 05′ 14.09″ W). This park is located near the center of the Serra do Mar Ecological Corridor (Aguiar et al. 2005) and is one of the principal remnants of Atlantic Forest in the state of Rio de Janeiro.

Capture of the rodents

Rodents were captured in November 2014 and July 2015 along ten transects in each expedition. The specimens were captured in Tomahawk® (16×5×5 in.) and Sherman® traps set on the ground, a total of 90 live traps set per day. Additionally, 20 pitfall traps, made of 100-l buckets, were buried in the ground, along four transects, a capture effort of 80 pitfalls traps set per day. Each trapping session lasted for ten consecutive days. All animals were captured alive and euthanized a posteriori for sample collection. Voucher specimens were deposited in the scientific collection of the National Museum (Museu Nacional) at the Universidade Federal do Rio de Janeiro (UFRJ). All the samples were collected during the Rede Bioma (Biome Network) project “Inventories: Patterns of Diversity, Biogeography, and Endemism of Mammals, Birds, Amphibians, Fruit Flies, and Parasites in the Atlantic Forest,” which is supported by a consortium of Brazilian research agencies, financed by CNPq PPBio Rede Bioma.

Collection and morphological analyses of oocysts

Samples were collected during necropsy, directly from the large intestine. The fecal material was placed in a 15-ml conical centrifuge tube containing a 2.5% (w/v) solution of K$_2$Cr$_2$O$_7$ and maintained at room temperature. On five consecutive days, the tube was opened and the solution was stirred vigorously to oxygenate it and promote the sporulation of any oocyst present in the faces. The samples were processed in a saturated sugar solution by the centrifugal flotation method (Sheather’s method), placed on slides examined under a microscope at a magnification of × 400 (Duszynski and Wilber 1997). Morphological observations and measurements were obtained using a Carl Zeiss Axio Scope.A1 binocular microscope with an apochromatic oil immersion objective lens and AxioVision imaging system. The oocysts were examined with an Imager.A2 light microscope equipped with Nomarski interference contrast microscopy × 100 objective lenses, and the images captured with an AxioCam MRc. All measurements are given as the mean value in micrometers, followed by the range of values in parentheses.

Histopathological analysis

To determine the site of infection, the small intestines of specimens positive for oocysts in their feces were segmented and fixed overnight in Carson Millonig formalin. The tissue samples were subsequently dehydrated in a progressive series of ethyl alcohol concentrations, diaphonized using xylol, and
then embedded in paraffin blocks for the preparation of serial sections of 5 μm. The tissue sections were stained with hematoxylin and eosin and examined under a light microscope Carl Zeiss (Humason 1979). The developmental stages were photographed and measured, and then analyzed qualitatively and described based on the scheme of Kheysin (2013).

**Results**

Four (7.5%) of the 53 *A. montensis* specimens analyzed were infected by an undescribed form of *Eimeria*. The histological analyses revealed the endogenous stages of the parasite in the small intestine of the mice, including the presence of macrogametocytes, microgametocytes, zygotes, and oocysts.

*Eimeria akodonensis* n. sp.

*Type host:* *Akodon montensis* Thomas, 1913 (Rodentia: Sigmodontinae), Symbiotype host (Frey et al. 1992), skin and skeleton, deposited in the National Museum of Rio de Janeiro (adult males, MNRJ nos. 83768, 83774, 83776, 83777).

*Type-locality:* Serra dos Órgãos National Park in Petrópolis, in the state of Rio de Janeiro, Brazil (22° 27′ 49″ S, 43° 05′ 14.09″ W).

*Type-material:* The oocysts were preserved in 70% ethanol, based on Duszynski and Gardner (1991). The samples were deposited in the Parasite Collection of the Department of Animal Parasitology (http://r1.ufrj.br/lcc) at the Federal Rural University of Rio de Janeiro, in Seropédica, Rio de Janeiro.

Fig. 4 Composite line drawing of the sporulated oocyst of *Eimeria akodonensis* n. sp. Scale bar = 10 μm

Fig. 5–12 Light micrographs of the endogenous stages of *Eimeria akodonensis* n. sp. observed in the lamina propria of the small intestine of *Akodon montensis*, showing the microgamonts (Mi) and the parasitophorous vacuole in the host cell (Vp); microgametes (Mi); macrogamonts (Ma); nucleus (N) and the zygote (Zy) with wall-forming bodies arranged around its periphery (Ow); oocyst (Oo). Scale bar = 10 μm
Janeiro, Brazil. Phototypes and line drawings were deposited together with the specimens. The catalog number is P-77/2017. Sporulation time: Unknown. Site of infection: Small intestine. Prevalence: 4 of 53 (7.5%). Etymology: The specific epithet is derived from the name of the host genus.

Exogenous stage
Description (Figs. 1–3 and 4)
Sporulated oocyst
Oocyst shape (n = 126) is ellipsoidal to subspherical, wall bi-layered, 1.5 μm (1.3–1.6) thick, outer layer rough. Oocyst length is 25.3 μm (21.0–28.0), with a width of 20.2 μm (17.0–22.0) and length/width (L/W) ratio of 1.3 μm (1.2–1.4). Polar granule is present, with oocyst residuum as a large spherical to subspherical globule.

Sporocyst
Sporocyst shape (n = 126) is ellipsoidal with length of 11.8 μm (9.3–14.4), width of 7.9 μm (6.7–9.3), and (L/W) ratio of 1.5 μm (1.4–1.7). Sporocysts with nipple-like Stieda body and sub-Stieda body are absent. A sporocyst residuum formed by several globules, usually along the sporocyst wall.

Endogenous stages
Description (Fig. 5–12)
The histological analysis revealed the endogenous development of the parasite in the jejunum portion of the small intestine. The infected cells of the lamina propria contained only parasites in the gametogenic phase, with the immature microgametocytes being enveloped by the parasitophorous vacuole, and having a rounded shape, approximately 10.9 μm (5.4–16.8) in length and 11.8 μm (5.5–17.3) in width (Fig. 5). Free immature microgametocytes and microgametes were also observed (Figs. 6–7), as were macrogametes at different stages of development, including the formation of the oocyst wall from the granules, approximately 20.9 μm (15.9–25.7) in length and 16.2 μm (12.6–19.9) in width, subspherical to ellipsoidal in shape (Figs. 8–10). Mature and immature oocysts were also observed (Figs. 11–12).

Discussion
In Brazil, the coccidian diversity found in wild rodents is still poorly known, since only three species were reported infecting rodents of the subfamily Sigmodontinae (Rodentia: Cricetidae): Eimeria oryzomysi from specimens of Oryzomyini rodents (Carini 1937), Eimeria zygodontomyis in Necromys lasiurus (Lainson and Shaw 1990), and Besnoitia akodoni in Akodon montensis (Dubey et al. 2003). In Venezuela, Eimeria akodoni was described in the grass mouse Necromys urichi J. A. Allen and Chapman, 1897 (Arcay-de-Peraza 1981), while Eimeria ojastii
As reported by Arcay-de-Peraza (1964), the morphology and morphometry of *Eimeria akodonensis* n. sp. were compared with data for coccidia of the family Eimeriidae described from other cricetid hosts in Brazil and Venezuela (Table 1). The various characteristics of *E. akodonensis* n. sp. were compared with the characteristic of *Eimeria ojastii* (Arcay-de-Peraza, 1964), *Eimeria yzygodontonymysis* (Lainson and Shaw 1990) and *Eimeria oryzomysi* (Arcay-de-Peraza, 1964) in order to study the presence of three walls in the oocysts of *E. akodonensis* n. sp., but slightly different in their morphology and size (14 × 12 μm vs. 25.3 × 20.2 μm) and both have a polar granule, although the sporocysts are slightly different in their morphology and size (14 μm × 7 μm vs. 11.8 μm × 7.9 μm). Arcay-de-Peraza (1981) describes the oocysts of *E. akodonii* as fusiform, and when they were observed in immersion, their thick wall was apparent along their whole length except for the extremities, where they become thinner, taking on the appearance of “false operculae,” in the words of the author. This characteristic is not observed in *Eimeria akodonensis* n. sp., and the oocysts are not fusiform in shape. Arcay-de-Peraza describes the presence of three walls in *E. akodonii* and the absence of residuum in the oocyst, whereas in *Eimeria akodonensis* n. sp., the oocyst has a double wall, with a large residuum. Despite these broad morphological similarities, the endogenous development of *Eimeria akodonensis* n. sp. occurs in different portions of the intestine in comparison with *E. akodonii*. Whereas *Eimeria akodonensis* n. sp. develops in the small intestine, Arcay-de-Peraza (1981) found *E. akodonii* in the large intestine. According to Arcay-de-Peraza (1981), merogony and gametogony of *E. akodonii* occurred in epithelial cells of the large intestine. In the case of *E. akodonensis* n. sp., gametogony was only recorded in the jejunum portion of the small intestine. The microgametocytes of *E. akodonensis* n. sp. were smaller (10.9 × 11.8 μm) than *E. akodonii* (30.6 × 20.4 μm). In addition, macrogametocytes of *E. akodonensis* n. sp. were sub-spherical, 20.9 × 16.2 μm (L/W ratio: 1.2 μm), and those of *E. akodonii* were ellipsoidal, 19.7 × 11.7 μm (L/W ratio: 1.6 μm). The effects of infection by *Eimeria akodonensis* n. sp. on the intestinal tissue of *Akodon montensis* varied considerably, from a total absence to a light and diffuse infiltration distributed in the intestinal mucosa composed of neutrophils and mononuclear cells. However, this infiltration was not found in association with the parasites themselves, but rather in the epithelium of the intestinal villi. Overall, the set of morphological and physiological characteristics found in *Eimeria akodonensis* n. sp. supports conclusively its description as a new species.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All trapping procedures followed the guidelines for the capture, handling, and care of animals established by the Ethics Committee on the Use of Animals in Research of Oswaldo Cruz Foundation/Fiocruz, Rio de Janeiro, under license numbers L-049/08 and LW81/12. The capture of wild animals was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through authorization number 45839-1, based on the pertinent Brazilian legislation.

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