

# A new coccidian, *Isospora parnaitatiaiensis* n. sp. (Apicomplexa, Eimeriidae), from the white-shouldered fire-eye *Pyriglena leucoptera* (Passeriformes, Thamnophilidae) from South America

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**Abstract** A new coccidian species (Protozoa: Apicomplexa: *Isospora*) parasitizing the white-shouldered fire-eye *Pyriglena leucoptera* (Vieillot, 1818) is described in the Parque Nacional do Itatiaia. This park is a protected area in southeastern Brazil with a high degree of vulnerability, representing a “conservation island” of biodiversity. *Isospora parnaitatiaiensis* n. sp. has oocysts that are ellipsoidal, 23.8×19.4 μm, with smooth, bilayered wall, ~1.1 μm thick. Micropyle and oocyst residuum are absent, but one or two polar granules are present. Sporocysts are ellipsoidal, 14.6×9.3 μm. The Stieda body is nipple- to knob-like and sub-Stieda body rounded to rectangular. Sporocyst residuum is present, usually as a cluster of numerous granules. Sporozoites are vermiform with two refractile bodies and a nucleus. This is the second isosporoid coccidian described from antbirds (Thamnophilidae).

**Keywords** Oocysts · Coccidia · Morphology · Passerines · Parque Nacional do Itatiaia · Conservation · Brazil

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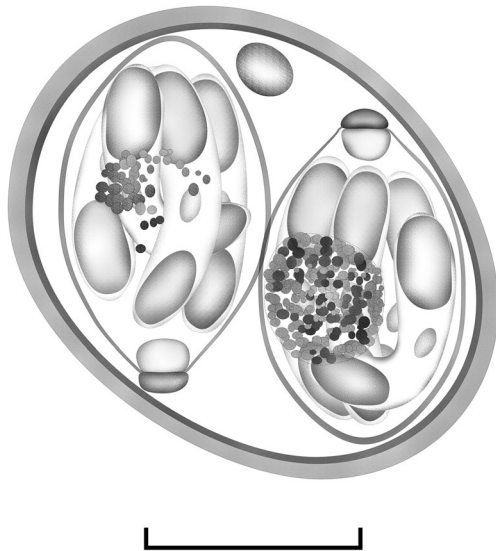
## Introduction

The Thamnophilidae family is comprised of small passerines often observed capturing ants and other arthropods in dense vegetation. Because of this behavior, they are named “antbirds” (Wiens 1989). The South American forest on the Atlantic coast and the Amazon are two major distribution areas of the Thamnophilidae family (Zimmer and Isler 2003).

The white-shouldered fire-eye *Pyriglena leucoptera* (Vieillot, 1818) is common in the understory of tropical moist forests, secondary forests, and forest edges and occurs from the state of Bahia to Rio Grande do Sul, in Brazil, as well as the east of Paraguay and Misiones, Argentina (Sick 1997; BirdLife International 2012).

The Parque Nacional do Itatiaia (PNI) is located in the Serra da Mantiqueira on the border of the states of Rio de Janeiro, Minas Gerais, and São Paulo. It is a protected area with a high degree of vulnerability and is considered a “conservation island” of biodiversity (ICMBIO 2015).

To date, only one coccidian species had been reported from Thamnophilidae. *Isospora sagittulae* McQuiston & Capparella, 1992 was originally described from the spotted antbird *Hylophylax naevioides* (Lafresnaye, 1847) (McQuiston and Capparella 1992) collected in the rain forests of northwestern Ecuador. Recently, this same isosporoid species was reported from the white-throated antbird *Gymnopathys salvini* (Berlepsch, 1901) and from the common scale-backed antbird *Willisornis poecilinotus* (Cabanis, 1847) in the Brazilian Amazon. These reports demonstrate the occurrence of this parasite in non-sympatric birds of the same family but from different genera that inhabit opposite sides of the Andes and of the Amazon River (Berto et al. 2014a).



**Fig. 1** Line drawing of *Isospora parnaitataiensis*, a new coccidium species recovered from the white-shouldered fire-eye *Pyriglena leucoptera*. Scale bar 10  $\mu$ m

The aim of this study was to examine the feces from white-shouldered fire-eyes *P. leucoptera* to determine what coccidian parasites were present. These *P. leucoptera* specimens were captured in and around the PNI boundaries, in southeastern Brazil.

## Materials and methods

A total of four sample periods were conducted in and around the PNI boundaries. Sampling occurred in August (22° 25' 46" S, 44° 37' 17" W), November (22° 26' 57" S, 44° 36' 25" W), December 2014 (22° 27' 20" S, 44° 36' 28" W), and March 2015 (22° 27' 38" S, 44° 35' 34" W). A total of eight *P. leucoptera* were captured. The birds were kept in individual boxes and feces collected immediately after defecation. After identification of the species, the bird was released and stool samples were placed in centrifuge tubes containing a potassium dichromate 2.5 % ( $K_2Cr_2O_7$ ) solution at 1:6 (v/v). Samples were carried to the Laboratório de Coccídios e Coccidioses, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature for

10 days or until ~70 % of the oocysts were sporulated. Oocysts were isolated by flotation in Sheather's sugar solution (specific gravity, 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014b). Morphological observations, line drawings, photomicrographs, and measurements were made using an Olympus BX binocular microscope coupled to a digital camera Eurocam 5.0. Line drawings were edited using two software applications from CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), specifically Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometers and are given as the range followed by the mean in parentheses.

## Results

Eight *P. leucoptera* were captured and examined, and three were positive for coccidia. All observed oocysts were characteristic of *Isospora*, which is described and named as follows:

***Isospora parnaitataiensis* n. sp.** (Figs. 1 and 2a–c).

**Type-host:** White-shouldered fire-eye *Pyriglena leucoptera* (Vieillot, 1818) (Aves: Passeriformes: Thamnophilidae).

**Type-specimens:** Oocysts in 70–100 % ethanol, phototypes and line drawings are deposited and available (<http://r1.ufrj.br/lcc>) in the Parasitology Collection of the Laboratório de Coccídios e Coccidioses, at UFRRJ, located in Seropédica, Rio de Janeiro, Brazil. Photographs of the type-host specimens (symbiotypes) are deposited in the same collection. The repository number is P-59/2015.

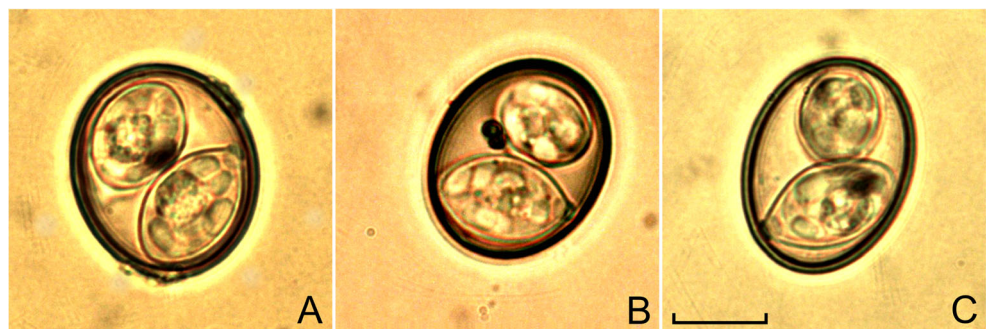
**Type-locality:** Parque Nacional do Itatiaia (22° 27' S, 44° 35' W), southeastern Brazil.

**Site of infection:** Unknown.

**Etymology:** The specific epithet is a homage to the Parque Nacional do Itatiaia, which is the first national park in Brazil (founded in 1937).

**Description of the sporulated oocyst:** Oocyst ( $n=23$ ) ellipsoidal, 22–27  $\times$  18–21 (23.8  $\times$  19.4); length/width (L/W) ratio 1.1–1.5 (1.23). Wall bi-layered, 1.0–1.2 (1.1) thick, outer layer smooth,  $c.2/3$  of total thickness. Micropyle and oocyst residuum both absent, but one or two polar granules are present.

**Fig. 2** Photomicrographs (a–c) of sporulated oocysts of *Isospora parnaitataiensis*, a new coccidium species recovered from the white-shouldered fire-eye *Pyriglena leucoptera*. Scale bar 10  $\mu$ m



*Description of the sporocyst and sporozoites:* Sporocysts ( $n=22$ ) 2, ellipsoidal,  $13-16 \times 8-10$  ( $14.6 \times 9.3$ ); L/W ratio 1.5–1.7 (1.6). Stieda body present, nipple-like to knob-like, 1.0 high  $\times$  2.0 wide; sub-Stieda present, rounded to rectangular, 1.5 high  $\times$  2.0 wide; para-Stieda body absent; sporocyst residuum present, usually as a distinctly ovoidal to ellipsoidal body consisting of numerous small granules that appear to be membrane-bounded,  $5-6 \times 4-5$  ( $5.5 \times 4.5$ ). Sporozoites 4, vermiform, with anterior and posterior refractile bodies and centrally located nucleus.

**Discussion**

The PNI is characterized by mountains and rocky terrain with elevation ranging from 600 to 2791 at the peak of Agulhas Negras (ICMbio 2015). The specimens of *P. leucoptera* were captured at all four sample sites at altitudes ranging from 600 to 1,200 m indicating that this antbird species is widely distributed in the PNI and a potential disperser of coccidia to other antbird species (Berto and Lopes 2013).

The results of this study reveal that the oocysts of *I. parnaitaiensis* are noticeably polymorphic in the characteristics of the sporocyst, Stieda and sub-Stieda bodies. In some oocysts, the sporocysts were observed more (40 %) (Fig. 2c) and in others less (60 %) (Fig. 2b) elongated. The Stieda body was observed sometimes as nipple-like (50 %) (Fig. 2a) and in others knob-like (50 %) (Fig. 2b). The sub-Stieda body was observed sometimes as rounded (85 %) (Fig. 2a) and in others rectangular (15 %) (Fig. 2b).

This polymorphism in the oocysts exposes the importance and complexity of identifying coccidian species. Currently, the identification of coccidia from Passeriformes has been based on different criteria by different authors. For example, prior to 1982, more than 100 species of passerines had been reported as hosts of *Isospora lacazei* (Labbé, 1893). Therefore, based on available descriptions and on the improbability that a single species could parasitize so many hosts, Levine (1982) suggested the names *Isospora passeris* Levine, 1982 for parasites of *Passer domesticus* Linnaeus, 1758 and *I. lacazei* for parasites of *Carduelis carduelis* Linnaeus, 1758. However, Al-Quraishy and Al-Nasr (2009) discussed whether all coccidia from passerines that were called *I. lacazei* were the same species. They concluded that diet influenced oocyst size in different passerines and that at least the species described from *P. domesticus* should be considered junior synonyms of *I. lacazei*. More recently, Dolnik et al. (2009) used a technique to isolate single oocysts and extract DNA for PCR and sequencing and found six different haplotypes of *Isospora* from wild birds. They found both the same haplotype in different host individuals and various combinations of haplotypes in the

**Table 1** Comparative morphology of *Isospora* spp. recorded from New World antbirds (Thamnophilidae)

Coccidia	Hosts	References	Oocysts				Sporocysts						
			Shape	Measurements (µm)	Shape index	Wall	Polar granule	Shape	Measurements (µm)	Shape index	Stieda body	Sub-Stieda body	Residuum
<i>Isospora sagittulae</i> McQuiston & Capparella, 1992	<i>Hylophylax naevoides</i> (Lafresnaye, 1847)	McQuiston and Capparella (1992)	Ovoidal to ellipsoidal	25–30 $\times$ 21–24 (27.5 $\times$ 21.8)	1.27	Bi-layered	Present, 1–3	Subspherical to ovoidal	13–16 $\times$ 12–13 (14.8 $\times$ 12.4)	1.19	Present, thin and dense	Present, triangular	Diffuse
	<i>Gymnophis savini</i> (Berlepsch, 1901); <i>Willsonis poecilnotus</i> (Cabanis, 1847)	Berto et al. (2014a)	Ovoidal to ellipsoidal	27–31 $\times$ 20–24 (28.4 $\times$ 22.4)	1.27	Bi-layered	Present, 1–3	Subspherical to ovoidal	13–17 $\times$ 12–14 (15.0 $\times$ 12.6)	1.2	Thin and flattened, 0.5 $\times$ 2.0	Triangular to rounded, 2.5 $\times$ 5.0	Diffuse
<i>Isospora parnaitaiensis</i> n. sp.	<i>Pyrgilena leucoptera</i> (Vieillot, 1818)	Current work	Ellipsoidal	22–27 $\times$ 18–21 (23.8 $\times$ 19.4)	1.23	Bi-layered	Present, 1–2	Ellipsoidal	13–16 $\times$ 8–10 (14.6 $\times$ 9.3)	1.6	Nipple-like to knob-like, 1.0 $\times$ 2.0	Rounded to rectangular, 1.5 $\times$ 2.0	Compact

same host individual. When they found oocysts that belonged to four different haplotypes in the feces of one passerine, they concluded that mixed infections with different haplotypes are rather common in wild birds. Finally, Duszynski and Wilber (1997) suggest that a new coccidian species should be compared by morphology and morphometry of the oocysts in detail with coccidian species that are feature-similar and belong to the same host family. In this sense, they put forward the concept of intra-familial specificity, which was reaffirmed by Berto et al. (2011) in New World passerines.

We agree with Al-Quraishy and Al-Nasr (2009) that there should be synonyms in some species of coccidia of passerines, especially those described in the same host at different times and conditions and without detailed description. In contrast, the idea that hundreds of *Isospora* spp. (distinct and identifiable morphologically) belong to a single species is inconsistent and may well be an obstacle to our knowledge of the coccidia of passerines.

At another extreme, the concept that slight genetic variation is sufficient to differentiate species brings confusion and an exponential amplification of the number of existing species. For example, Hafeez et al. (2014) described two new species from starlings *Lamprotornis superbus* Rüppell, 1845. In their work, they observed the same extra-intestinal stages and the same oocyst morphology. However, they obtained two distinct COI sequences (1.7 % divergence) and two distinct 18S rDNA sequences (0.6 % divergence), which they used as their basis for naming two new species. These findings do not approach with studies of other protozoa, as *Toxoplasma gondii* Dubey & Beattie, 1988 and *Paramecium* spp. which have more prudent and consistent criteria and approaches. *T. gondii* has a genetic divergence of 2–3 % in North American and European genotypes, with the exception of those ones of South America, which have much more genetic diversity due to older and hybridized population (Khan et al. 2014). In a study of the genetic diversity of *Paramecium* spp. using the COI gene, an intra-specific variation of 11 % was obtained, or rather, specimens with up to 11 % of genetic divergence were considered as the same species (Zhao et al. 2013). In this sense, we understand that small genotypic variations favored the polymorphism observed in oocysts of this work, although these morphological changes have been observed in sporocysts in a single oocyst. Therefore, if genetic variations actually exist, they should not be sufficient to describe other species, but genotypes within a single species. Moreover, Grulet et al. (1982) and Berto et al. (2014b) suggest that small variations in Stieda and sub-Stieda bodies may be a result of the sporulation process, the position of the sporozoite inside the sporocyst, or the position of their oocysts and sporocysts under the coverslip at the time of evaluation.

Thus, we opted to compare our new species with coccidian species that are feature-similar and belong to the same host family (Duszynski and Wilber 1997). The only other coccidian

species described in Thamnophilidae is *I. sagittulae*, which was originally described from *H. naevioides* in Ecuador and, recently, reported from the Amazonian antbirds *G. salvini* and *W. poecilinotus* (McQuiston and Capparella 1992; Berto et al. 2014a).

*I. sagittulae* (Table 1) differed from *I. parnaitatiaiensis* in having larger oocysts with sporocyst subspherical to ovoidal and Stieda body thin and flattened. Therefore, this species is easily distinguished from *I. parnaitatiaiensis*.

This finding emphasizes how little we know about the parasites of New World passerines. There are 186 Thamnophilidae species in Brazil, and so far, only three antbird species have been examined for coccidia and all have been found infected with at least one *Isospora*. Antbirds have wide geographic ranges (Berto et al. 2014a). Thus, our work encourages further studies to examine all other species in the family to determine the true coccidian fauna of this most interesting family of birds.

Based on the morphological features described above, *I. parnaitatiaiensis* is considered as new to science and the second isosporoid coccidian reported from a thamnophilid bird.

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