

## ***Isospora pitiguari* n. sp. (Apicomplexa: Eimeriidae) from the rufous-browed peppershrike (Aves: Passeriformes: Vireonidae) *Cyclarhis gujanensis* Gmelin, 1789**

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### **Abstract**

In the current study, a new coccidian species (Protozoa: Apicomplexa: Eimeriidae), collected from the rufous-browed peppershrike *Cyclarhis gujanensis* Gmelin, 1789, is reported from Brazil. *Isospora pitiguari* n. sp. has oocysts, which are spherical to sub-spherical, 26.8 × 25.7 µm, with smooth, bilayered wall ~1.5 µm thick. Micropyle, oocyst residuum, and polar granule are absent. Sporocysts are rounded to slightly ovoidal, 14.4 × 11.6 µm. Stieda body flattened and substieda body prominent and rounded. Sporocyst residuum is composed of granules of different sizes. Sporozoites are vermiform with one refractile body and a nucleus. This is the first description of an isosporoid coccidium infecting a New World vireo.

**Key words:** taxonomy, morphology, coccidia, *Isospora*, oocysts, Passeriformes, Vireonidae, Marambaia Island, Rio de Janeiro, Brazil

### **Introduction**

The rufous-browed peppershrike *Cyclarhis gujanensis* Gmelin, 1789 is a New World vireo. It is widespread and often common in woodland, forest edge, and cultivation with some tall trees from Mexico and Trinidad south to Argentina and Uruguay (Sick 1997; CBRO 2011; IUCN, 2013).

Boughton *et al.* (1938) recovered *Isospora*-like oocysts from feces of red-eyed vireos *Vireo olivaceus* Linnaeus, 1766. These oocysts were obtained from captured vireos in zoos, but no species were described or named. After this report, coccidia have never been reported from vireos. However, according Cicero & Johnson (2001) and CBRO (2011), this family is phylogenetically closely related to the Corvidae and Meliphagidae, into Parvorder Corvida, from which four distinct isosporoid species have been described to date (Berto *et al.*, 2011).

The current study describes the first coccidian species infecting the rufous-browed peppershrike *C. gujanensis*, a New World vireo, on Marambaia Island, Rio de Janeiro State, Brazil.

### **Material and methods**

One rufous-browed peppershrike was captured using nets in Marambaia Island (23°04'S, 43°53'W). The bird was kept for 10–20 min in an individual cage and feces collected immediately after defecation. After identification of the bird species, the bird was released and the fecal samples were placed in plastic vials containing 2.5% potassium dichromate solution ( $K_2Cr_2O_7$ ) 1:6 (v/v). Samples were sent to the Laboratório de Coccídios e Coccidioses,

Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were placed in a thin layer (~5 mm) of  $K_2Cr_2O_7$ , 2.5% solution in Petri plates and incubated at 23–28°C for 10 days or until ~70% of the oocysts were sporulated. Oocysts were recovered by flotation in Sheather's sugar solution (Specific gravity: 1.20) and examined microscopically using the technique described by Duszynski & Wilber (1997). Morphological observations and measurements, given in micrometers ( $\mu m$ ), were made using a Carl Zeiss binocular microscope with an apochromatic oil immersion objective lens and an ocular micrometer (K-15X PZO, Poland). Line drawings were prepared using a Wild M-20 binocular microscope with a drawing tube. Micrographs were taken using a digital camera (Sony model CD Mavica MVC-CD250). Size ranges are shown in parenthesis after average and shape index (L/W ratio). Abbreviations: total number of measurements [n], micropyle [M], oocyst residuum [OR], polar granule [PG], Stieda body [SB], substieda body [SSB], parastieda body [PSB], sporocyst residuum [SR], sporozoite [SZ], refractile body [SRB], nucleus [N].

## Results

The captured rufous-browed peppershrike was a healthy male and shed oöcysts of one species. Initially, the oöcysts were non-sporulated, but 70% were sporulated by day four.

### *Isospora pitiguary n. sp.*

**Type host:** Rufous-browed peppershrike *Cyclarhis gujanensis* Gmelin, 1789 (Aves: Passeriformes: Vireonidae).

**Type locality.** Marambaia Island (23°04'S, 43°53'W), Rio de Janeiro, Brazil.

**Site of infection.** Not investigated.

**Type-material.** One-half of the oocysts were kept in 10% aqueous buffered formalin (v/v) and the other half were stored in 70% ethanol according Duszynski & Gardner (1991). Both samples were deposited in the Parasitology Collection, in the Department of Animal Parasitology, at UFRRJ, located in Seropédica, Rio de Janeiro, Brazil. Phototypes and line drawings were deposited as well. The repository number is P-46/2013.

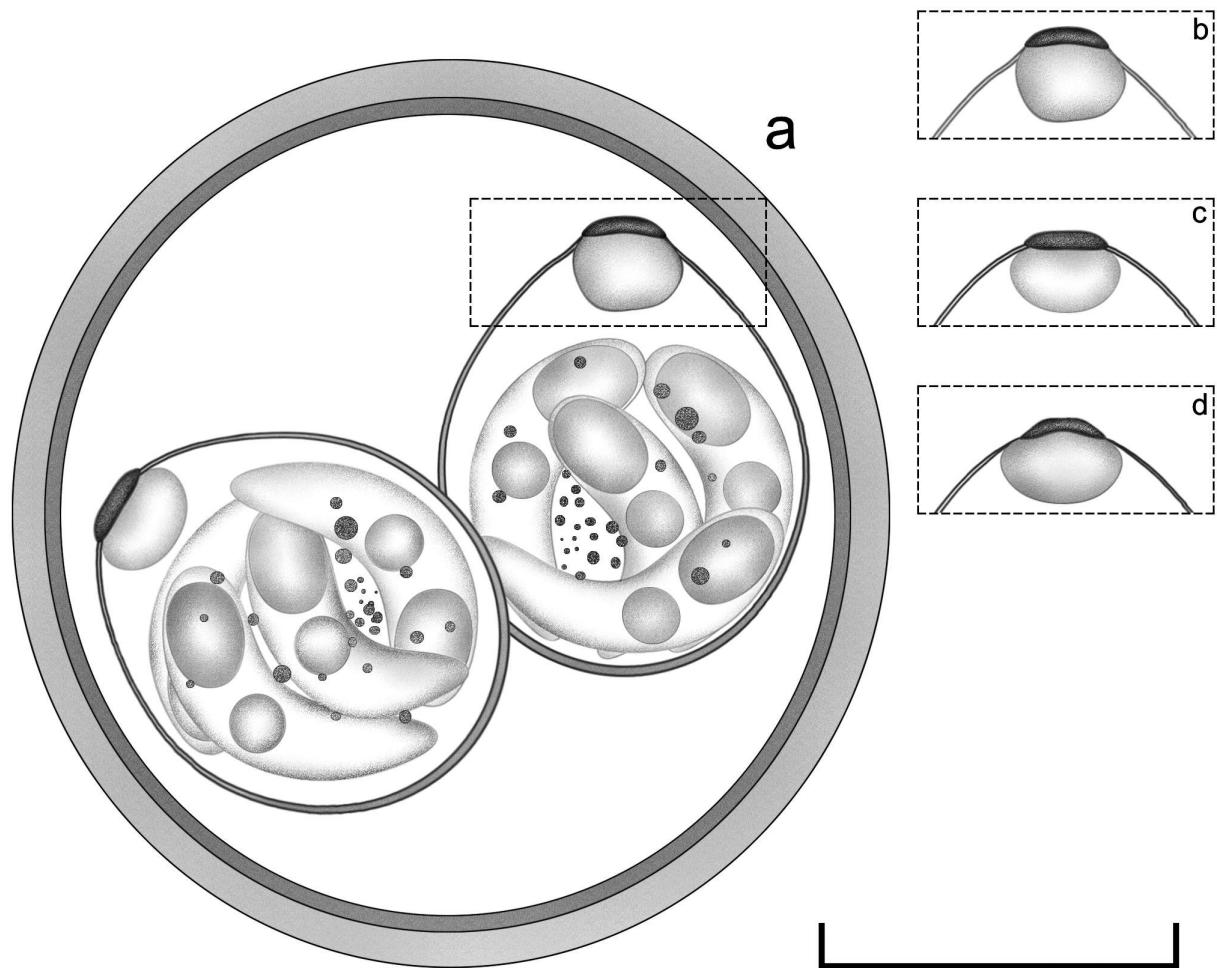
**Etymology.** The specific epithet is derived from the common local name for the host, which is 'pitiguari'.

**Description** (Figs 1a–c; 2a–c). Oocyst shape (n = 11) spherical to sub-spherical; oocyst wall bilayered; wall thickness 1.5 (1.3–1.6); outer wall smooth, c.2/3 of total thickness; L × W = 26.8 × 25.7 (23–28 × 23–28), L/W ratio = 1.0 (1.0–1.1); M, PG and OR: absent. Sporocyst shape (n = 11) rounded to slightly ovoidal; L × W = 14.4 × 11.6 (13–15 × 10–13); L/W ratio = 1.2 (1.2–1.3); SB present, flattened, 0.5 high × 2.5 wide; SSB present, prominent, rounded, 2.0 high × 3.0 wide; PSB absent; SR present, composed of granules of different sizes; SZ vermiform with 1 posterior SRB and centrally located N.

**Remarks.** *Isospora pitiguary* differs from other *Isospora* species from the passerines of same Parvorder (Table 1). In *I. pitiguary* lack PG, which is present in *Isospora brachyrhynchi* Wobester & Cawthorn, 1985, *Isospora cyanocoracis* Upton, Current & Clubb, 1985, *Isospora calocitta* Upton, Wright & Langen, 1995 and *Isospora samoensis* Adamczyk, McQuistion & LaPointe, 2004. Besides, these four species have elongated/ ovoidal sporocysts, whereas in *I. pitiguary* the sporocysts are sub-spherical (Berto *et al.* 2011).

## Discussion

Despite of the large range of *C. gujanensis* in the New World, this bird is rarely observed or captured at Marambaia Island. In 15 years of work by one of us (IF) in Marambaia Island, this is the first capture of this bird on the island. Therefore, only one fecal sample could be collected. Also for this reason, very few oocysts could be observed because only 50% of the single sample was processed, with the rest deposited in 10% aqueous buffered formalin and 70% ethanol. The 11 oocysts observed were carefully identified and measured to ensure the reliability of the description.



**FIGURE 1.** Line drawings of *Isospora pitiguary* n. sp., a new coccidium species recovered from the rufous-browed peppershrike *Cyclarhis gujanensis*. (a) sporulated oocyst with its respective variations of (b–d) Stieda and substieda bodies. Scale-bar: 10μm.



**FIGURE 2.** Photographs (a–b) of sporulated oocysts of *Isospora pitiguary* n. sp., a new coccidium species recovered from the rufous-browed peppershrike *Cyclarhis gujanensis*. Scale-bar: 10μm.

**TABLE 1.** Comparative morphology of *Isospora pitiguari* n. sp. and *Isospora* spp. recorded from Parvorder Corvida in New World.

Coccidia	Host	Reference	Oocysts				
			Shape	Measurements (μm)	Shape index	Wall (μm)	Polar granule
<i>I. brachyrhynchi</i>	<i>Corvus brachyrhynchos</i> Brehm, 1822 (Corvidae)	Wobester & Cawthorn (1985)	sub-spherical	20.4 × 18.9 (15–25 × 14–23)	1.1 (1.0–1.3)	c.1.0	present
<i>I. cyanocoracis</i>	<i>Cyanocorax chrysops</i> Vieillot, 1818 (Corvidae)	Upton et al. (1985)	sub-spherical	28.7 × 26.8 (25–30 × 24–29)	1.1 (1.0–1.1)	bi-layered, c.2.0	present, 1 or 2
<i>I. calocitta</i>	<i>Calocitta formosa</i> Swainson, 1827 (Corvidae)	Upton et al. (1995)	sub-spherical	28.8 × 27.7 (26–31 × 25–29)	1.0 (1.0–1.1)	bi-layered, c.2.0	present, 1 to 3
<i>I. samoensis</i>	<i>Foulehaio carunculatus</i> Gmelin, 1788 (Meliphagidae)	Adamczyk et al. (2004)	ovoid	28.9 × 26.1 (25–32 × 23–30)	1.1 (1.0–1.3)	bi-layered	present, 1 or 2
<i>I. pitiguari</i>	<i>Cyclarhis gujanensis</i> Gmelin, 1789 (Vireonidae)	Current study	sub-spherical	26.8 × 25.7 (23–28 × 23–28)	1.0 (1.0–1.1)	bi-layered, c.1.5	absent

continued.

Coccidia	Host	Reference	Sporocysts				
			Shape	Measurements (μm)	Stieda body	Substieda body	Residuum
<i>I. brachyrhynchi</i>	<i>Corvus brachyrhynchos</i> Brehm, 1822 (Corvidae)	Wobester & Cawthorn (1985)	elongate	16.2 × 10.6 (14–20 × 8–13)	present	—	diffuse
<i>I. cyanocoracis</i>	<i>Cyanocorax chrysops</i> Vieillot, 1818 (Corvidae)	Upton et al. (1985)	ovoid	19.3 × 11.4 (17–21 × 10–12)	prominent	homogeneous	compact
<i>I. calocitta</i>	<i>Calocitta formosa</i> Swainson, 1827 (Corvidae)	Upton et al. (1995)	ovoid	20.1 × 12.6 (19–22 × 11–14)	present	present	diffuse
<i>I. samoensis</i>	<i>Foulehaio carunculatus</i> Gmelin, 1788 (Meliphagidae)	Adamczyk et al. (2004)	ovoid	17.1 × 10.9 (16–18 × 10–11)	large	retangular	compact
<i>I. pitiguari</i>	<i>Cyclarhis gujanensis</i> Gmelin, 1789 (Vireonidae)	Current study	rounded to slightly ovoidal	14.4 × 11.6 (13–15 × 10–13)	flattened	rounded	diffuse

The collecting of symbiont hosts is important for future studies. However, in Brazil, the native birds are protected by law and supervised by IBAMA (the Brazilian Institute of the Environment and Natural Renewable Resources). Thus, it was not possible for us to archive the symbiont host.

According to Duszynski & Wilber (1997) and Berto et al. (2011), a new coccidian species from passerines should be morphologically compared with other congeneric coccidian species that share similar features and belong to the same host family. Therefore, despite of the unlikely transmission of coccidia between passerines of different families, due to the lack of descriptions of coccidian parasites from New World vireos, *I. pitiguari* was compared with the coccidia from birds of the families Corvidae and Meliphagidae, which are closely related to the Vireonidae.

Based on Table 1, it can be concluded that *I. pitiguari* is easily differentiated using the morphology and morphometry of the oocysts from *Isospora* species from New World passerines of same Parvorder. In addition, it is

possible that *I. pitiguari* is isolated on Marambaia Island along with its host *C. gujanensis* because the transmission between non-sympatric birds that inhabit distant continents, or islands is unlikely, as occurs with coccidia reported in Hawaii and American Samoa (Berto & Lopes, 2013). However, the Marambaia Island has a sand zone of around 40 km in extension (Marambaia Coastal Restinga), which is connected to the continent.

Finally, *I. pitiguari* is considered to be new to science, being the first description in a New World vireo.

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