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Original Article

Isospora bertoi n. sp. of the saffron finch, *Sicalis flaveola* (Aves: Passeriformes) from Brazil[1]

Isospora bertoi n. sp. do canário-da-terra, *Sicalis flaveola* (Aves: Passeriformes) do Brasil

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Abstract

A new species of coccidia (Protozoa: Apicomplexa: Eimeriidae) is described from the saffron finch, *Sicalis flaveola*, is reported from Brazil. Sporulated oocysts of *Isospora bertoi* n. sp. are spherical to subspherical; 23.6 (21.1-26.5) x 22.0 (19.4-24.6) µm; shape Index (L/W ratio) 1.1 (1.0-1.2) µm; with bilayer smooth walls, ~1.1 µm. Micropyle and oocyst residuum are absent, but polar granules are present. Sporocysts are elongated ellipsoidal, 16.2 (13.6-17.9) x 10.1 (8.9-12.4) µm. Stieda body is button-shaped and Sub-Stieda and Para-Stieda body are absent. Sporocyst residuum is compact and composed of hundreds of granules scattered among the sporozoites. The sporozoite is claviform with an elongated posterior refractile body and nucleus.

Keywords: *Isospora bertoi* n. sp., sporulated oocysts, saffron finch, coccidian.

Resumo

Uma nova espécie de coccídio (Protozoa: Apicomplexa: Eimeriidae) do canário-da-terra, *Sicalis flaveola*, é relatada no Brasil. Oocistos esporulados de *Isospora bertoi* n. sp. são esféricos a subesféricos; 23,6 (21,1-26,5) x 22,0 (19,4-24,6) µm com índice morfométrico (L/W) de 1,1 (1,0-1,2); com parede lisa constituída por duas camadas com ~1,1 µm. Micrópila e resíduo de oocisto estão ausentes, mas grânulos polares estão presentes. Os esporocistos são elipsoidais alongados, 16,2 (13,6-17,9) x 10,1 (8,9-12,4) µm;. O corpo Stieda é em forma de botão, enquanto que os corpos de Sub-Stieda e Para-Stieda estão ausentes. O resíduo do esporocisto é compacto e constituido por centenas de grânulos espalhados entre os esporozoítos. O esporozoíto é claviforme com corpo e núcleo refráteis posteriores alongados.

Palavras-chave: *Isospora bertoi* n. sp., oocistos esporulados, canário-da-terra, coccidio.

1. Introduction

The saffron finch, *Sicalis flaveola* (Linnaeus, 1766), is a bird belonging to the order Passeriformes. It has tiny dimensions and a more complex and developed syrinx, granting it greater song capacity and ability (Silveira and Méndez, 1999). Mainly a granivorous species, it also includes in its diet, fruits and insects that it obtains from the soil and herbaceous strata, branches and foliage up to five meters in height (Zotta, 1940; La Peña and Pensiero, 2003; La Peña, 2011). Its distribution extends from Central to South America, with introductions in Hawaii, Puerto Rico and Jamaica (Ridgely and Tudor, 1989).

Among the diseases that affect birds, coccidiosis is considered an important cause of enteritis and death of the species (Freitas et al., 2003; Coelho et al., 2011). According to Gallo et al. (2014) several species of Coccidia occur in the same species of bird, and most of these species are not pathogenic, demonstrating the importance of not only quantitatively assessing its diagnosis, but also qualitatively assessing it (Teixeira et al., 2014).

To date, two species of *Isospora* have been described in *S. flaveola* in captivity in the Municipality of Seropedica in the State of Rio de Janeiro, Brazil, and identified as *Isospora cetasiensis* (Coelho et al., 2011) and *Isospora sicalisi* (Coelho et al., 2011). The present study describes a new species of *Isospora* found in free-ranging *S. flaveola* with habitat in the Municipality of Eugenopolis in the State of Minas Gerais (MG), Brazil.

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2. Materials and Methods

Fecal samples were collected from 30 birds captured in a mist net in the Municipality of Eugenopolis (MG) Brazil. After capture, the birds were individually housed for 24 h in cages with water and food *ad libitum*. Feces found at the bottom of the cage were placed in 15 mL tubes, identified, placed in an isothermal box with ice and immediately transported to the Núcleo de Pesquisas Avançadas em Parasitologia (NUPAP) at the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) in the Municipality of Campos dos Goytacazes, RJ. All methods were performed in accordance with the relevant guidelines and regulations. Fecal samples were filtered through double gauze, mixed with 2.5% potassium dichromate ($K_2Cr_2O_7$), placed in each Petri dish and incubated at 23-28 °C until 70% of the oocysts were sporulated. Oocysts were retrieved by the flotation method with Sheather's sugar solution and examined microscopically using the method described by Duszynski and Wilber (1997).

Morphological observations and measurements obtained in micrometers were made by using an Eclipse i80 optical microscope (Nikon, Japan). Photomicrographs were taken using a Coolpix 5700 digital camera (Nikon, Japan) and drawings were made by superimposing images at different focuses using CorelDRAW software version 2020. Size ranges are given in parentheses, followed by the mean and Shape Index (SI) = Length/Width ratio.

3. Results

Thirty free-ranging saffron finches were captured, and oocysts with different morphological characteristics of *I. sicalisi* and *I. cetasiensis* were observed in the fecal samples of two (7%) of 30 birds. After the fourth day, more than 70% of these oocysts were fully sporulated under the conditions recommended in this study.

3.1. Isospora bertoi n. sp.

3.1.1. Host type: Sicalis flaveola (Aves: Passeriformes: Emberizidae)

Type material: Photosyntypes and linear drawings of sporulated oocysts are deposited and available in the Parasitology Collection at the Laboratório de Biologia de Coccídios in the Universidade Federal Rural do Rio de Janeiro, *Campus* Seropédica, Rio de Janeiro, Brazil. Photographs of the host specimens (symbiotypes) are deposited in the same collection. The repository number is P-126/2021.

3.1.2. Type locality: Eugenópolis (21°05'56" S and 42°11'13" W), Minas Gerais, Brazil

Prevalence: 2/30 (6.7%)

Sporulation time: 3 to 4 days at a temperature of 23 to 28 °C.

Site of infection: Unknown. Oocysts collected from fecal samples.

Etymology: The specific name is derived from the last name of Professor Bruno Pereira Berto, eminent protozoologist of the Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde Universidade Federal Rural do Rio de Janeiro (UFRRJ), *Campus* Seropédica, Rio de Janeiro, Brazil, granted in his honor for describing the two species of *Isospora* of *S. flaveola* and several other species of coccidia in birds.

3.2. Description

3.2.1. Sporulated oocyst

Subspherical to ellipsoid oocysts (n=32), 23.6 (21.1-26.5) x 22.0 (19.4-24.5), with a SI of 1.1 (1.0-1.2). Bi-layered wall, 1.1 (0.9-1.3) thick (n=18), smooth outer surface representing 2/3 of the total thickness; Micropyle and oocyst residuum absent; 1-2 polar granules of different shapes and sizes and can be bilobed (n=24), 2.9 (1.9-4.1) x 2.2 (1.1-3.6) (Figures 1 and 2).

3.2.2. Sporocyst and sporozoites

Sporocysts in elongated ellipsoid shape, tapering at both ends (n=37), 16.2 (13.6-17.9) x 10.1 (8.9-12.4) with SI 1.6 (1.3-1.9); Button-shaped Stieda body present, Sub-Stieda and para-Stieda bodies absent. Compact sporocyst residuum, composed of hundreds of granules scattered among the sporozoites. Claviform sporozoite with an elongated posterior refractile body and nucleus (Figures 1 and 2).

4. Discussion

Coccidia found in the present study were compared in detail with species reported in *S. flaveola* and other birds as suggested by Duszynski and Wilber (1997) for the description of a new species. In addition, they were compared with coccidia of New World passerine birds also belonging to the Emberizidae family, since there is a higher probability of transmission of this parasite among sympatric birds (Carvalho Filho et al., 2005; Berto et al., 2009).

Two species of *Isospora* have been described in *S. flaveola* in the State of Rio de Janeiro, Brazil previously (Coelho et al., 2011). *Isospora cetasiensis* differs from *I. bertoi* n. sp.; it does not possess a polar granule, its sporocyst is ovoidal with a rounded Sub-Stieda body and a diffuse residual body (Table 1). The oocyst of *I. sicalisi* has the largest size (27.5 x 25.2 µm) among the species described in *S. flaveola* and does not have a polar granule (Table 1). It has a trapezoidal Sub-Stieda body (Table 1), which was not observed in the new species described in this study, and the sporocyst residuum is diffuse, in contrast to the compact oocyst of *I. bertoi* n. sp.

In 1985, Upton et al. (1985) described *Isospora paroariae* (Upton et al., 1985) found in *Paroaria coronata* (Miller, 1776) (Red-crested cardinal) in Argentina. This species, in addition to having a much thicker oocyst wall than the species described in the present study, does not have a polar granule, and its sporocyst is ovoid with a prominent Sub-Stieda body.

In the Galapagos Islands in 1988, McQuistion and Wilson (1988) described four species of *Isospora* in *Camarhynchus parvulus* Gould, 1837 (Small tree finch) named *Isospora rotunda* (McQuiston and Wilson, 1988), *Isospora fragmenta* (McQuiston and Wilson, 1988), *Isospora exigua* (McQuiston and Wilson, 1988) and *Isospora temeraria* (McQuiston and Wilson, 1988).

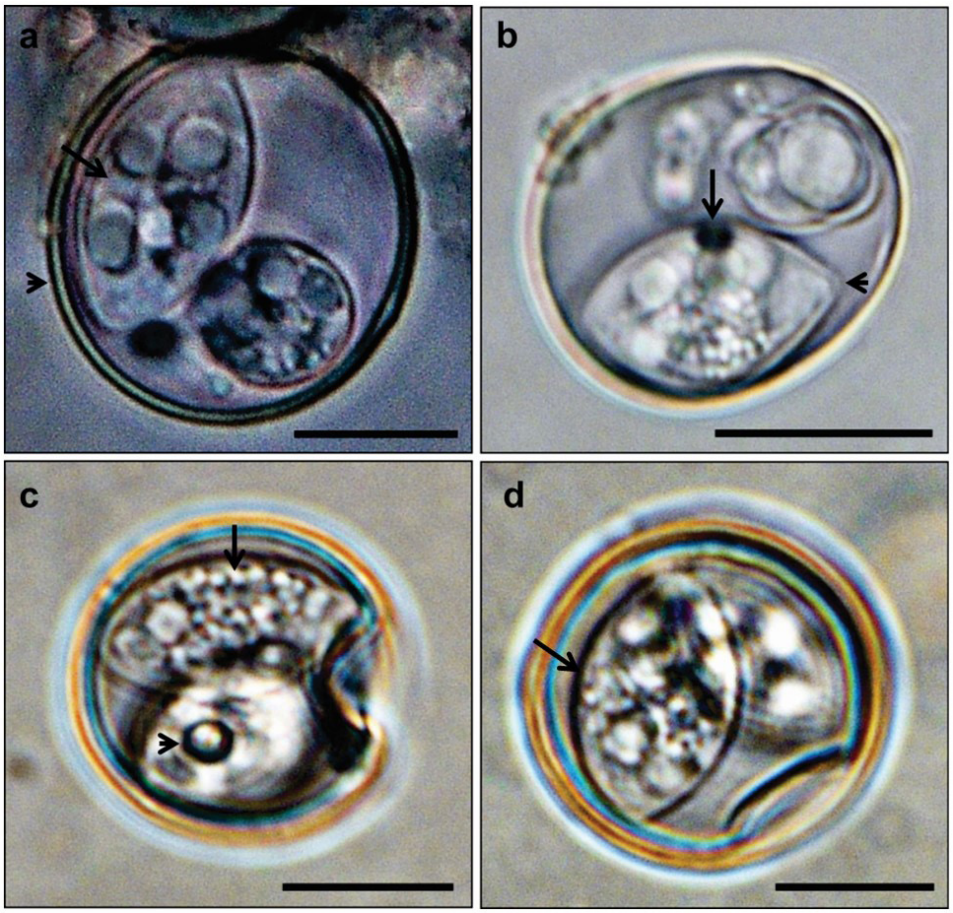

Figure 1. Photomicrographs of *Isospora bertoi* n. sp. of free-living saffron finch *Sicalis flaveola*. **a**, sporozoite (arrow) and bi-layered wall (arrowhead); **b**, polar granule (arrow) and Stieda body (arrowhead); **c**, sporocyst residuum (arrow) and polar granule (arrowhead); **d**, sporocyst (arrow). Scales: 10 µm.

All the described species have simple walls and sub-Stieda bodies, thus distinguishing them from *I. bertoi* n. sp. *Isospora exigua* has no polar granules, and its average size (20.4 x 20.1 µm) is smaller than that of *I. bertoi* n. sp. *Isospora fragmenta* and *I. temeraria* have polar granules of different shapes and in greater quantities when compared to the new species *Isospora* found in *S. flaveola* and described in this study.

The following year, the same authors described *Isospora geospizae* (McQuiston and Wilson, 1989) in *Geospiza fuliginosa* Gould, 1837 (Small ground finch) and *Geospiza fortis* Gould, 1837 (Medium ground finch) in the Galapagos Islands too. The oocyst (15.5 x 14.5 µm) and sporocyst (10 x 7.5 µm) of this species have a much smaller average size than *I. bertoi* n. sp., in addition to the oocyst having a simple wall and the sporocyst having a sub-Stieda body.

In 1990, in the Galapagos Islands, McQuistion (1990) described oocysts of the species *Isospora daphnensis* (McQuistion, 1990) in *G. fortis*. These oocysts, in addition to having average measurements (27.3 x 23.6 µm) larger than *I. bertoi* n. sp. also have the thickest wall (~1.5 µm). The sporocyst is ovoid with a mamiliform Stieda body and a small Sub-Stieda body.

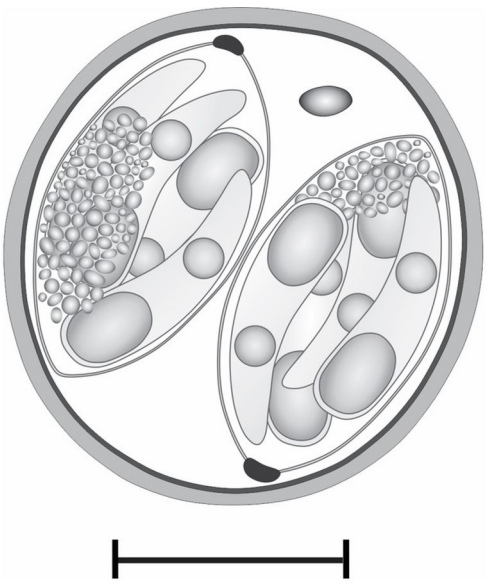

Figure 2. Schematic drawing of *Isospora bertoi* n. sp. of free-living saffron finch *Sicalis flaveola*. Bar: 10 µm.

Table 1. Comparative morphology and morphometry of *Isospora* spp. of American birds of the order Passeriformes, family Emberizidae.

Species	*Isospora bertoi*	*Isospora cetasiensis*	*Isospora sicalisi*	*Isospora paroariae*	*Isospora rotunda*	*Isospora fragmenta*	*Isospora exigua*	*Isospora temeraria*
Host	***Sicalis flaveola***	***Sicalis flaveola***	***Sicalis flaveola***	***Paroaria coronata***	***Camarhynchus parvulus***	***Camarhynchus parvulus***	***Camarhynchus parvulus***	***Camarhynchus parvulus***
Reference	**Present research**	**Coelho et al., 2011**	**Coelho et al., 2011**	**Upton et al., 1985**	**McQuistion and Wilson, 1988**	**McQuistion and Wilson, 1988**	**McQuistion and Wilson, 1988**	**McQuistion and Wilson, 1988**
Oocyst								
Shape	Subspherical to Ellipsoidal	Subspherical to Ellipsoidal	Subspherical to Ellipsoidal	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical	Ellipsoidal
Larger diameter	23.6 (21.1-26.5)	23.1 (19-27)	27.5 (25-29)	22.3 (19.5-25.5)	21.8 (20-24)	25.3(24-27)	20.4 (20-23)	25.4 (21-30)
Smaller diameter	22.0 (19.4-24.5)	21.6 (19-26)	25.2 (22-28)	21.4 (18.5-24.0)	20.9 (19-23)	24.2 (23-25)	20.1 (18-23)	21.1 (17-23)
Shape-index	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.1).	1.01	1.05	1.02	1.21
Wall	Bi-layered 1.1 (0.9-1.3)	Bi-layered 1.0 (0.7-1.2)	Bi-layered 1.1 (0.9-1.3)	Bi-layered ~1.8	One layer ~1.0	One layer ~1.0	One layer ~1.0	One layer ~1.0
Micropyle	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Polar granule	1-2 (varied formats)	Absent	Absent	Absent	Large	10-20 small splinter-like	Absent	1 large round
Oocyst residuum	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Sporocyst								
Shape	Elongated ellipsoid	Ovoid	Ellipsoid	Ovoid	Ovoid	Piriform	Ovoid	Piriform
Larger diameter	16.2 (13.6-17.9)	15.1 (13-19)	17.2 (15-19)	15.2 (14.0-16.5)	15 (13-16)	15.4 (14-17)	14 (13-15)	15 (14-15)
Smaller diameter	10.1 (8.9-12.4)	10.9 (10-13)	11.7 (11-12)	10.0 (8.0-11.5)	9.7 (9-10)	11.5 (11-12)	9.5 (8-10)	10 (9-11)
Shape-index	1.6 (1.3-1.9)	1.4 (1.3-1.6)	1.5 (1.3-1.6)	1.5 (1.3-1.7)	1.55	1.34	1.47	1.50
Stieda body (Width x Height)	Button-shaped	Knob-like (2.5x1.0)	Knob-like (2.0x1.5)	2.3 x 0.8	knob-like	knob-like	Small	knob-like
Sub-Stieda body (Width x Height)	Absent	Rounded (2.5x1.5)	Trapezoidal (6.0x2.5)	Prominent (3.3 x 1.7)	Prominent	Prominent	Small	Prominent
Sporocyst residuum	Granular Compact	Granular Diffuse	Granular Diffuse	Granular Spherical or subspherical,	Round, well consolidated	Granular irregular-shaped	Granular irregular-shaped	Granular Round consolidated
Sporozoite								
Larger diameter	-	-	-	11.3 (10.0-13.5)	-	-	-	-
Smaller diameter	-	-	-	3.4 (3.2-4.0)	-	-	-	-
Refractile body	1	1	1	1	2	2	2	2
Nucleus	Present	Present	Present	Not discernible	-	-	-	-

Table 1. Continued...

Species	*Isospora bertoi*	*Isospora geospizae*	*Isospora daphnensis*	*Isospora tiaris*	*Isospora sporophilae*	*Isospora flausinoi*	*Isospora teixeirafilhoi*	*Isospora curio*
Host	*Sicalis flaveola*	*Geospiza fuliginosa*	*Geospiza fortis*	*Asemospiza fuliginosa*	*Sporophila caerulescens*	*Sporophila caerulescens*	*Sporophila caerulescens*	*Oryzoborus angolensis*
Reference	**Present research**	**McQuistion and Wilson, 1989**	**McQuistion, 1990**	**Ball and Daszak, 1997**	**Carvalho Filho et al., 2005**	**Carvalho Filho et al., 2005**	**Carvalho Filho et al., 2005**	**Silva et al., 2006**
Oocyst								
Shape	Subspherical to Ellipsoidal	Spherical to Subspherical	Ellipsoidal	Subspherical	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical
Larger diameter	23.6 (21.1-26.5)	15.5 (13-17)	27.3 (22-30)	27.1 (24.7-30)	21.6 (19.2-23.2)	17.3 (14-20)	17.4 (1.6-19.4)	24.6 (22-26)
Smaller diameter	22.0 (19.4-24.5)	14.5 (12-17)	23.6 (20-27)	23.8 (21.2-26.5)	20.9 (18.4-22.6)	16.5 (13.6-20)	16.8 (14.2-18.8)	23.6 (22-25)
Shape-index	1.1 (1.0-1.2)	1.0 (1.0-1.1)	1.1 (1.0-1.3)	1.1	1.0 (1-1.10)	1.0 (1-1.2)	1.0 (1-1.1)	1.0 (1.0-1.1)
Wall	Bi-layered 1.1 (0.9-1.3)	One layer ~1.0	Bi-layered ~1.5	Bi-layered ~1.0	Bi-layered 1.3	Bi-layered 1.0	Bi-layered 1.2	Bi-layered ~1.5
Micropyle	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Polar granule	1-2 (varied formats)	1 ovoid	Present	1 Ellipsoidal	Splinter-like or comma-like	1 Large	Small double-lobuled	Absent
Oocyst residuum	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Sporocyst								
Shape	Elongated ellipsoid	Ovoid	Ovoid	Ovoid	Ovoid	Piriform	Ovoid	Ovoid
Larger diameter	16.2 (13.6-17.9)	10 (10-12)	15.2 (15-16)	14.7 (12.4-16.8)	15.1 (12.8-17.4)	14.9 (11.8-18)	11.7 (9-14.2)	13.2 (15-17)
Smaller diameter	10.1 (8.9-12.4)	7.5 (6-9)	10.2 (9-11)	10.8 (8.8-12.4)	10.6 (8.4-12.6)	10.7 (8-12.4)	8.1 (6.2-9.4)	10.9 (10-13)
Shape-index	1.6 (1.3-1.9)	1.3 (1.2-1.5)	1.5 (1.4-1.7)	1.4	1.4 (1.2-1.8)	1.4 (1.1-1.8)	1.5 (1.1 -1.9)	1.6 (1.4-1.7)
Stieda body (Width x Height)	Button-shaped	Small rounded	Nipple-like	Prominent	knob-like	Rounded	knob-like	Present (2.0-2.5 x 0.7)
Sub-Stieda body (Width x Height)	Absent	Small	Small	Prominent	Absent	Absent	Absent	Absent
Sporocyst residuum	Granular Compact	Granular Irregular-shaped	Granular Compact	Granular Irregular-shaped	Granular Irregular-shaped	Granular Compact	Granular Compact	Granular Diffuse
Sporozoite								
Larger diameter	-	-	-	-	-	-	-	18.2 (16-19)
Smaller diameter	-	-	-	-	-	-	-	3.5 (3-4)
Refractile body	1	2	1	1	1	1	1	2
Nucleus	Present	-	-	-	-	-	-	Present

Table 1. Continued...

Species	*Isospora bertoi*	Isospora braziliensis	*Isospora paranaensis*	*Isospora frontalis*	*Isospora teresopoliensis*	*Isospora chanchaoi*	*Isospora ticoticoi*
Host	***Sicalis flaveola***	***Oryzoborus angolensis***	***Oryzoborus angolensis***	***Sporophila frontalis***	***Sporophila frontalis***	***Sporophila frontalis***	***Zonotrichia capensis***
Reference	**Present research**	**Silva et al., 2006**	**Silva et al., 2006**	**Berto et al., 2009**	**Berto et al., 2009**	**Berto et al., 2009**	**Balthazar et al., 2009**
Oocyst							
Shape	Subspherical to Ellipsoidal	Spherical to Subspherical	Subspherical to elliptical	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical	Spherical to Subspherical
Larger diameter	23.6 (21.1-26.5)	17.8 (16-19)	24.3 (22-26)	27.9 (27–29)	25.7 (24–27)	24.2 (23–26)	23.3 (20–25)
Smaller diameter	22.0 (19.4-24.5)	16.9 (16-18)	19.8 (18-22)	26.9 (25–28)	24.3 (23–25)	22.0 (21–23)	22.4 (20–24)
Shape-index	1.1 (1.0-1.2)	1.1 (1.0-1.1)	1.2 (1.1-1.4)	1.0 (1.0–1.1)	1.1 (1.0–1.1)	1.1 (1.0–1.1)	1.0 (1.0–1.1)
Wall	Bi-layered 1.1 (0.9-1.3)	Bi-layered ~1.0	One layer ~1.5	Bi-layered 1.4 (1.3–1.5)	Bi-layered 1.3 (1.2–1.4)	Bi-layered 1.2 (1.1–1.2)	Bi-layered 1.2 (1.1–1.3)
Micropyle	Absent	Absent	Absent	Absent	Absent	Absent	
Polar granule	1-2 (varied formats)	Absent	1 Ellipsoid	Splinter-like or comma shaped	Absent	1–2	Usually absent
Oocyst residuum	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Sporocyst							
Shape	Elongated ellipsoid	Ellipsoid	Ovoid	Ellipsoid	Ovoid	Ellipsoid	Ellipsoid
Larger diameter	16.2 (13.6-17.9)	13.2 (12-14)	15.7 (14-18)	19.6 (19–21)	18.8 (18–20)	6.1 (15–17)	17.0 (15–18)
Smaller diameter	10.1 (8.9-12.4)	10.8 (9-12)	10.1 (8-12)	11.1 (10–12)	11.2 (10–12)	10.3 (10–11)	10.8 (10–11)
Shape-index	1.6 (1.3-1.9)	1.5 (1.3-1.6)	1.5 (1.3-1.7)	1.8 (1.6–1.9)	1.7 (1.6–1.7)	1.6 (1.5–1.6)	1.6 (1.5–1.7)
Stieda body (Width x Height)	Button-shaped	Fine plug (~ 1 wide)	Present (2.0-2.5 x 1.5)	Knob-like (1.7 x 0.8)	Nipple-shaped (1.9 x 0.8)	Nipple-shaped (2 x 0.8)	Nipple-shaped (2 x 1)
Sub-Stieda body (Width x Height)	Absent	Absent	Present (2-2.5 x 2-2.5)	Slight	Large, Prominent (3.7 x 2.2)	Small, Prominent (2.2 x 1.4)	Prominent, Compartmentalized (2.4 x 1.5)
Sporocyst residuum	Granular Compact	Granular Diffuse	Granular Diffuse	Granular Diffuse	Granular Diffuse	Granular Compact	Granular Diffuse
Sporozoite							
Larger diameter	-	13.2 (12-14)	14.9 (13-16)	-	-	-	-
Smaller diameter	-	3.1 (2.5-3.5)	3.0 (2-4)	-	-	-	-
Refractile body	1	2	2	1	1	1	1
Nucleus	Present	Present	Present	Present	Present	Present	Present

Table 1. Continued...

Species	*Isospora bertoi*	*Isospora bocamontensis*	*Isospora nigricollis*
Host	***Sicalis flaveola***	***Gubernatrix cristata***	***Sporophila nigricollis***
Reference	**Present research**	**Pereira et al., 2011**	**Barreto, 2014**
Oocyst			
Shape	Subspherical to Ellipsoidal	Subspherical	Spherical to Subspherical
Larger diameter	23.6 (21.1-26.5)	32.1 (27–34)	18 (16-20)
Smaller diameter	22.0 (19.4-24.5)	28.9 (26–32)	17 (15-20)
Shape-index	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1 (1 – 1.1)
Wall	Bi-layered 1.1 (0.9-1.3)	Bi-layered 1.5 (1-2)	Bi-layered ~1.0
Micropyle	Absent	Absent	Absent
Polar granule	1-2 (varied formats)	1 ellipsoidal	Absent
Oocyst residuum	Absent	Absent	Absent
Sporocyst			
Shape	Elongated ellipsoid	Ellipsoid	Ellipsoid
Larger diameter	16.2 (13.6-17.9)	17.3 (16-19)	12 (12-13)
Smaller diameter	10.1 (8.9-12.4)	12.2 (11-13)	8
Shape-index	1.6 (1.3-1.9)	1.4 (1.3-1.5)	1.5
Stieda body (Width x Height)	Button-shaped	Half-moon-shaped (2.3 x 0.7)	Flattened
Sub-Stieda body (Width x Height)	Absent	Prominent, homogeneous (3.7 x 2.1)	Absent
Sporocyst residuum	Granular Compact	Granular Compact	Granular Diffuse
Sporozoite			
Larger diameter	-	-	-
Smaller diameter	-	-	-
Refractile body	1	1	1
Nucleus	Present	Present	Present

In 1997, in Venezuela, Ball and Daszak (1997) described *Isospora tiaris* (Ball and Daszak, 1997) oocysts in *Asemospiza fuliginosa* (Sooty grassquit) with mean measurements (27.1 x 23.8 µm) larger than *I. bertoi* n. sp. The sporocysts are ovoid with a prominent Sub-Stieda body and diffuse residuum, characteristics that differ from the oocysts described in the present study.

Three new species of *Isospora* were described in *Sporophila caerulescens* (Vieillot, 1823) (double-collared seedeater) by Carvalho Filho et al. (2005) in 2005 in the State of Rio de Janeiro, Brazil. Oocysts of these species, named *Isospora sporophilae*, *Isospora flausinoi* and *Isospora teixeirafilhoi* (Carvalho Filho et al., 2005), are smaller than those of *I. bertoi* n. sp. The sporocysts of these species have different shapes from those reported in the new species *Isospora* described here. Similar to oocysts, sporocysts also have lower mean values, especially *I. teixeirafilhoi*, which has very small sporocysts (11.7 x 8.1 µm).

Silva et al. (2006), described three new species of *Isospora* found in *Oryzoborus angolensis* (Chestnut-bellied seed finch) in the State of Mato Grosso do Sul, Brazil. The oocyst wall of *Isospora curio* (Silva et al., 2006) is thicker than that of *I. bertoi* n. sp. and it does not have polar granules. The sporocysts are ovoid and have much smaller average sizes (13.2 x 10.9 µm) than those described in *S. flaveola* in the present research, and the sporocyst residuum is diffuse. The oocysts of *Isospora braziliensis* (Silva et al., 2006) are smaller (17.8 x 16.9 µm) than those of *I. bertoi* n. sp., as well as sporocysts (13.2 x 10.8 µm). The oocyst wall is simple, polar granules are absent, and sporocyst residuum is diffuse. *Isospora paranaensis* (Silva et al., 2006) oocysts also have a single wall, and their average thickness is approximately 1.5 µm. The sporocysts are ovoid and have a Sub-Stieda body.

In 2009, Berto et al. (2009), described three new species of *Isospora* in *Sporophila frontalis* (Berto et al., 2009) (Buffy-fronted seedeater) from Teresopolis in the State of Rio de Janeiro, Brazil. The oocysts (27.9 x 26.9 µm) and sporocysts (19.6 x 11.1 µm) of *Isospora frontalis* have larger mean sizes than those found in *I. bertoi* n. sp. The oocyst wall differs from that of *I. bertoi* n. sp. because it is thicker (~1.4 µm) and the polar granules are comma-shaped. Other features, such as the presence of a Sub-Stieda body and diffuse sporocyst residuum differentiate these species. The species *Isospora teresopoliensis* (Berto et al., 2009) differs from *I. bertoi* n. sp. by the lack of polar granules and by the presence of a Sub-Stieda body and diffuse sporocyst residuum. The oocysts of *Isospora chanchaoi* (Berto et al., 2009) differ morphologically from the species described in the present study, with the presence of a Sub-Stieda body, a sporocyst different in shape and compact sporocyst residuum, but with granules of a different shape, size and distribution.

Balthazar et al. (2009) described the species *Isospora ticoticoi* in *Zonotrichia capensis* (Berto et al., 2009) (Rufous-collared sparrow) also in the Municipality of Teresópolis in the State of Rio de Janeiro, Brazil. It has a compartmentalized Sub-Stieda body and diffuse oocyst residuum, which was not observed in *I. bertoi* n. sp.

Oocysts of *Isospora bocamontensis* were described in *Gubernatrix cristata* (Pereira et al., 2011) (Yellow Cardinal) by Pereira et al. (2011) at the Municipality of Santa Maria in the State of Rio Grande do Sul, Brazil. The oocysts of this species are considerably larger (32.1 x 28.9 µm) than those described in *I. bertoi* n. sp. in addition to having a thicker wall (~1.5 µm). The sporocysts have a different shape from that observed in *I. bertoi* n. sp. and a prominent Sub-Stieda body, which was not observed in the species described in this study.

In 2014, the oocysts of *Isospora nigricollis* (Barreto, 2014) were described in *Sporophila nigricollis* (Yellow-bellied Seedeater) at the Municipality of Belo Horizonte in the State of Minas Gerais, Brazil by Barreto (2014). Both oocysts (18 x 17 µm) and sporocysts (12 x 8 µm) have much smaller dimensions than those observed in *I. bertoi* n. sp. In addition, the oocysts do not have a polar granule, and the sporocyst is not composed of residuum.

Based on morphological and morphometric characteristics, *Isospora bertoi* is considered a new species not previously described. It is the third species of *Isospora* described in *S. flaveola*.

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