

Two new *Isoospora* species from the saffron finch, *Sicalis flaveola* in Brazil

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Abstract

Two new coccidian species (Protozoa, Apicomplexa, Eimeriidae) are reported from the saffron finch *Sicalis flaveola* Linnaeus, 1766, a very common species in South America. *Isoospora cetasiensis* sp. nov. oocysts are subspherical to ellipsoidal, $23.1 \times 21.6 \mu\text{m}$, with smooth, bilayered wall, $\sim 1.0 \mu\text{m}$. Micropyle, oocyst residuum and polar granule are absent. Sporocysts are ovoidal, $15.1 \times 10.9 \mu\text{m}$. Stieda body is knob-like and substieda body is rounded. Sporocyst residuum is composed of many scattered granules and spherules of different sizes. Sporozoites are vermiform with one refractile body and a nucleus. *Isoospora sicalisi* sp. nov. oocysts are subspherical to ellipsoidal, $27.5 \times 25.2 \mu\text{m}$, with a smooth, bilayered wall, $\sim 1.1 \mu\text{m}$. Micropyle, oocyst residuum and polar granule are absent. Sporocysts are ellipsoidal, $17.2 \times 11.7 \mu\text{m}$. Stieda body is knob-like and substieda body is trapezoidal. Sporocyst residuum is composed of scattered granules and spherules of different sizes. Sporozoites are vermiform with one refractile body and a nucleus.

Keywords

Coccidia, *Isoospora cetasiensis* sp. nov., *I. sicalisi* sp. nov., oocysts, Passeriformes, Emberizidae, South America

Introduction

The saffron finch *Sicalis flaveola* Linnaeus, 1766 is an emberizid bird from South America. It has a wide distribution in Argentina, Aruba, Bolivia, Brazil, Cayman Islands, Colombia, Ecuador, French Guiana, Guyana, Netherlands Antilles, Panama, Paraguay, Peru, Puerto Rico, Suriname, Trinidad and Tobago, Uruguay and Venezuela. This species has always been of great interest to people due to their bold colors and vocal repertoire and, for these reasons, has been illegally captured and traded in Brazil (Stotz *et al.* 1996, Sick 1997, CBRO 2009).

The current study describes two coccidian species infecting saffron finches *S. flaveola* recovered from illegal trade. These specimens were held in CETAS/IBAMA (Centro de Triagem de Animais Silvestres – Center for Triagem of Wild Animals) for rehabilitation and reintroduction into the wild.

Materials and methods

Fecal samples were collected from 26 birds held in individual cages in CETAS/IBAMA, located at the municipality of Seropédica in the State of Rio de Janeiro, Brazil. Feces were collected immediately after defecation and placed in plastic vials containing 2.5% potassium dichromate solution ($\text{K}_2\text{Cr}_2\text{O}_7$) 1:6 (v/v). Samples were carried to the Laboratório de Coccídios e Coccidioses located at the Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were placed in a thin layer (c. 5 mm) of 2.5% $\text{K}_2\text{Cr}_2\text{O}_7$ solution in Petri dishes and incubated at $23^\circ\text{--}28^\circ\text{C}$ for 10 days or until 70% of the oocysts were sporulated. Oocysts were recovered by flotation in Sheather's sugar solution (S.G. 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997). Morphological observations and measurements, given in micrometers, were made using a Carl Zeiss

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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. Photomicrographs were taken using a digital camera (Sony CD Mavica MVC-CD250). Size ranges are in parentheses following the means. 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), length (L) and width (W).

Results

Twenty-six saffron finches were examined; 18 of them shed oocysts in the feces. Initially, the oocysts were non-sporulated, 70% sporulated by day four.

Isospora cetasiensis sp. nov. (Figs 1a-d, 3a-b)

Description of sporulated oocyst: Oocyst shape (n = 48): sub-spherical to ellipsoidal; number of walls 2; wall thickness 1.0 (0.7–1.2); outer wall smooth, about 2/3 of total thickness; L ×

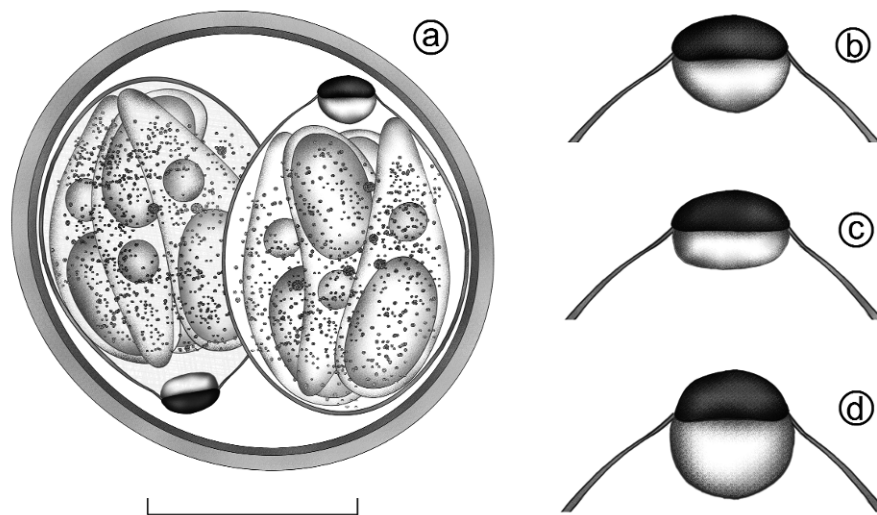


Fig. 1. Line drawings of *Isospora cetasiensis*, a new coccidian species recovered from the saffron finch *Sicalis flaveola*: (a) sporulated oocyst with its respective variations of (b-d) Stieda and substieda bodies detached. Scale bar = 10 μ m for oocysts and 5 μ m for Stieda and substieda bodies

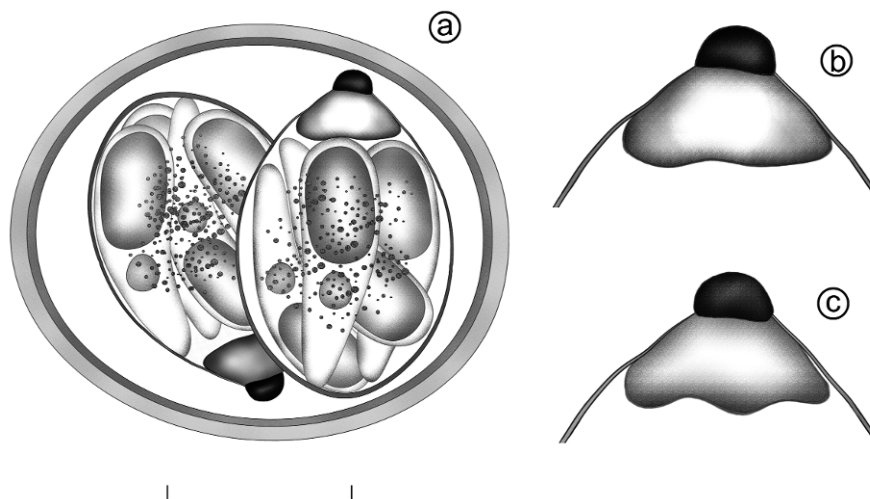


Fig. 2. Line drawings of *Isospora sicalisi*, a new coccidian species recovered from the saffron finch *Sicalis flaveola*: (a) sporulated oocyst with its respective variations of (b, c) Stieda and substieda bodies detached. Scale bar = 10 μ m for oocysts; and 5 μ m for Stieda and substieda bodies

Table I. Comparative morphology of *Isoospora* spp. recorded from New World emberizid birds

Coccidia	Host	References	Oocysts				Sporocysts					
			shape	measurements (µm)	shape index	wall	polar granule	shape	measurements (µm)	Stieda body	substieda body	residium
<i>Isoospora paroariae</i> Upton, Current et Clubb, 1985	<i>Paroaria coronata</i> Miller, 1776	Upton <i>et al.</i> (1985)	subspherical	22.3 × 21.4 (19-26 × 18-24)	1.1 (1.0-1.1)	bi-layered, ~1.8	absent	ovoidal	15.2 × 10.0 (14-17 × 8-12)	small	prominent	compact
<i>I. rotunda</i> McQuiston et Wilson, 1988	<i>Camarhynchus parvulus</i> Gould, 1837	McQuiston & Wilson (1988)	subspherical	21.8 × 20.9 (20-24 × 19-23)	1.0	one-layered, ~1.0	present	ovoidal	15 × 9.7 (13-16 × 9-10)	knob-like	prominent	compact
<i>I. fragmenta</i> McQuiston et Wilson, 1988	<i>C. parvulus</i> Gould, 1837	McQuiston & Wilson (1988)	subspherical	25.3 × 24.2 (24-27 × 23-25)	1.1	one-layered, ~1.0	present, 10 to 20	pyriform	15.4 × 11.5 (14-17 × 11-12)	knob-like	prominent	compact
<i>I. exigua</i> McQuiston et Wilson, 1988	<i>C. parvulus</i> Gould, 1837	McQuiston & Wilson (1988)	subspherical	20.4 × 20.1 (20-23 × 18-23)	1.0	one-layered, ~1.0	absent	ovoidal	14 × 9.5 (13-15 × 8-10)	small	small	compact
<i>I. temeraria</i> McQuiston et Wilson, 1988	<i>C. parvulus</i> Gould, 1837	McQuiston & Wilson (1988)	ellipsoidal	25.4 × 21.1 (21-30 × 17-23)	1.2	one-layered, ~1.0	present, 1 to 4	pyriform	15 × 10 (14-15 × 9-11)	knob-like	prominent	compact
<i>I. geospizae</i> McQuiston et Wilson, 1989	<i>Geospiza fortis</i> Gould, 1837	McQuiston & Wilson (1989)	subspherical	15.5 × 14.5 (13-17 × 12-17)	1.1 (1.0-1.1)	one-layered, ~1.0	present	ovoidal	10 × 7.5 (10-12 × 6-9)	rounded	small	compact
<i>I. daphnensis</i> McQuiston, 1990	<i>G. fortis</i> Gould, 1837	McQuiston (1990)	ellipsoidal	27.3 × 23.6 (22-30 × 20-27)	1.2 (1.0-1.3)	bi-layered, ~1.5	present	ovoidal	15.2 × 10.2 (15-16 × 9-11)	nipple-like small	small	compact
<i>I. tiaris</i> Ball et Daszak, 1997	<i>Tiaris fuliginosa</i> Wied, 1830	Ball & Daszak (1997)	subspherical	27.1 × 23.8 (25-30 × 21-27)	1.1	bi-layered, ~1.0	present	ovoidal	14.7 × 10.8 (12-17 × 9-12)	prominent	prominent	diffuse
<i>I. sporophilae</i> Carvalho-Filho, Meireles, Ribeiro et Lopes, 2005	<i>Sporophila caerulea</i> Vieillot, 1823	Carvalho-Filho <i>et al.</i> (2005)	subspherical	21.6 × 20.1 (19-23 × 18-23)	1.0 (1.0-1.1)	bi-layered, ~1.3	present	ovoidal	15.1 × 10.7 (13-17 × 8-13)	knob-like	absent	compact
<i>I. flausinoi</i> Carvalho-Filho, Meireles, Ribeiro et Lopes, 2005	<i>S. caerulea</i> Vieillot, 1823	Carvalho-Filho <i>et al.</i> (2005)	subspherical	17.3 × 16.5 (14-20 × 14-20)	1.1 (1.0-1.2)	bi-layered, ~1.0	present	pyriform	14.9 × 10.7 (12-18 × 8-12)	rounded	absent	compact
<i>I. teixeirafilhoi</i> Carvalho-Filho, Meireles, Ribeiro et Lopes, 2005	<i>S. caerulea</i> Vieillot, 1823	Carvalho-Filho <i>et al.</i> (2005)	subspherical	17.4 × 16.8 (16-19 × 14-19)	1.0 (1.0-1.2)	bi-layered, ~1.2	present	ovoidal	11.7 × 8.1 (9-14 × 6-9)	knob-like	absent	compact
<i>I. curio</i> Silva, Literák et Koudela, 2006	<i>Oryzoborus angolensis</i> Linnaeus, 1766	Silva <i>et al.</i> (2006)	subspherical	24.6 × 23.6 (22-26 × 22-25)	1.0 (1.0-1.2)	bi-layered, ~1.5	absent	ovoidal	13.2 × 10.9 (15-17 × 10-13)	present	absent	diffuse
<i>I. brazilensis</i> Silva, Literák et Koudela, 2006	<i>O. angolensis</i> Linnaeus, 1766	Silva <i>et al.</i> (2006)	subspherical	17.8 × 16.9 (16-19 × 16-18)	1.1 (1.0-1.1)	one-layered, ~1.0	absent	ellipsoidal	13.2 × 10.8 (12-14 × 9-12)	flattened	absent	diffuse
<i>I. paranaensis</i> Silva, Literák et Koudela, 2006	<i>O. angolensis</i> Linnaeus, 1766	Silva <i>et al.</i> (2006)	subspherical to ellipsoidal	24.3 × 19.8 (22-26 × 18-22)	1.2 (1.1-1.4)	one-layered, ~1.5	present	ovoidal	15.7 × 10.1 (14-18 × 8-12)	present	present	compact
<i>I. frontalis</i> Berto, Balhazar, Flausino et Lopes, 2009	<i>S. frontalis</i> Verreaux, 1869	Berto <i>et al.</i> (2009)	subspherical	27.9 × 26.9 (27-29 × 25-28)	1.0 (1.0-1.1)	bi-layered, ~1.4	present	ellipsoidal	19.6 × 11.1 (19-21 × 10-12)	knob-like	delicated	diffuse

<i>I. teresopolensis</i> Berto, Balthazar, Flausino et Lopes, 2009	<i>S. frontalis</i> Verreaux, 1869	Berto <i>et al.</i> (2009)	subspherical	25.7 × 24.3 (24-27 × 23-25)	1.1 (1.0-1.1)	bi-layered, ~1.3	absent	ovoidal	18.8 × 11.2 (18-20 × 10-12)	nipple-like	large	diffuse
<i>I. chanchaoi</i> Berto, Balthazar, Flausino et Lopes, 2009	<i>S. frontalis</i> Verreaux, 1869	Berto <i>et al.</i> (2009)	subspherical or ovoidal	24.2 × 22.0 (23-26 × 21-23)	1.1 (1.0-1.1)	bi-layered, ~1.2	present, 1 or 2	ellipsoidal	16.1 × 10.3 (15-17 × 10-11)	nipple-like	small	compact
<i>I. ticoicoi</i> Balthazar, Berto, Flausino et Lopes, 2009	<i>Zonotrichia capensis</i> Muller, 1776	Balthazar <i>et al.</i> (2009)	subspherical	23.3 × 22.4 (20-25 × 20-24)	1.1 (1.0-1.1)	bi-layered, ~1.2	usually absent	ellipsoidal	17.0 × 10.8 (15-18 × 10-11)	nipple-like	compartimented	diffuse
<i>I. bocamontensis</i> Pereira, Berto, Flausino, Lovato et Lopes, 2011	<i>Gubernatrix cristata</i> Vieillot, 1817	Pereira <i>et al.</i> (2011)	subspherical	32.1 × 28.9 (27-34 × 26-32)	1.0 (1.0-1.2)	bi-layered, ~1.5	usually absent	ellipsoidal	17.3 × 12.2 (16-19 × 11-13)	half-moon-shaped	prominent	compact
<i>I. cetasiensis</i> sp. nov.	<i>Sicalis flaveola</i> Linnaeus, 1766	current work	subspherical to ellipsoidal	23.1 × 21.6 (19-27 × 19-26)	1.1 (1.0-1.2)	bi-layered, ~1.0	absent	ovoidal	15.1 × 10.9 (13-19 × 10-13)	knob-like	rounded	diffuse
<i>I. sicalisi</i> sp. nov.	<i>Sicalis flaveola</i> Linnaeus, 1766	current work	subspherical to ellipsoidal	27.5 × 25.2 (25-29 × 22-28)	1.1 (1.0-1.2)	bi-layered, ~1.1	absent	ellipsoidal	17.2 × 11.7 (15-19 × 11-12)	knob-like	trapezoidal	diffuse

W 23.1 × 21.6 (19–27 × 19–26), with L/W ratio 1.1 (1.0–1.2); M, OR and PG absent.

Description of sporocyst and sporozoites: Sporocyst shape (n = 48): ovoidal; L × W 15.1 × 10.9 (13–19 × 10–13); L/W ratio 1.4 (1.3–1.6); SB present, knob-like, 1.0 high × 2.5 wide; SSB present, rounded, 1.5 high × 2.5 wide; PSB absent; SR present, and composed of many scattered granules and spherules of different sizes; SZ vermiform with 1 posterior SRB and a nucleus.

Type-host: The saffron finch *Sicalis flaveola* Linnaeus, 1766 (Passeriformes, Emberizidae).

Type-locality: Seropédica (22°43'23.79"S and 43°42'36.94"W) Rio de Janeiro, Brazil.

Material deposited: Oocysts stored in 10% aqueous buffered formalin (v/v), and deposited in the Parasitology Collection, in the Department of Animal Parasitology, at UFRRJ, located in Seropédica, Rio de Janeiro, Brazil. Phototypes and line drawings are deposited at the same location. The repository number is P-38/2011.

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

Prevalence: 69% (18 of 26 examined birds).

Etymology: The specific epithet is a homage to the CETAS/IBAMA that is located at the municipality of Seropédica in the State of Rio de Janeiro, Brazil.

Remarks: *Isoospora cetasiensis* has oocysts with similar dimensions to the oocysts described by Upton *et al.* (1985), Ball and Daszak (1997), Carvalho-Filho *et al.* (2005), Silva *et al.* (2006), Berto *et al.* (2009), Balthazar *et al.* (2009). These species: *Isoospora tiaris* Ball, Daszak, 1997; *Isoospora sporophylae* Carvalho-Filho, Meireles, Ribeiro et Lopes, 2005; *Isoospora paranaensis* Silva, Literák et Koudela, 2006 and *Isoospora chanchaoi* Berto, Balthazar, Flausino et Lopes, 2009 have PG; *Isoospora paroariae* Upton, Current et Clubb, 1985 has compact SR and prominent SSB; *Isoospora curio* Silva, Literák et Koudela, 2006 has no SSB; *Isoospora teresopolensis* Berto, Balthazar, Flausino et Lopes, 2009 has nipple-like SB and large SSB; and *Isoospora ticoicoi* Balthazar, Berto, Flausino et Lopes, 2009 has nipple-like SB and a rounded to elongated SSB. All these characteristics were not found in *I. cetasiensis* (Table I).

Isoospora sicalisi sp. nov. (Figs 2a-c, 3c-d)

Description of sporulated oocyst: Oocyst shape (n = 15) subspherical to ellipsoidal; number of walls 2; wall thickness 1.1 (0.9–1.3); outer wall smooth, about 2/3 of total thickness; L × W 27.5 × 25.2 (25–29 × 22–28), with L/W ratio 1.1 (1.0–1.2); M, OR and PG absent.

Description of sporocyst and sporozoites: Sporocyst shape (n = 15): ellipsoidal; L × W 17.2 × 11.7 (15–19 × 11–12); L/W ratio 1.5 (1.3–1.6); knob-like SB, 1.5 high × 2.0 wide; trapezoidal shaped SSB with an irregular base occasionally, 2.5 high × 6.0 wide; no PSB but SR present, composed of scattered granules and spherules of different sizes; SZ vermiform with 1 posterior SRB and a nucleus.

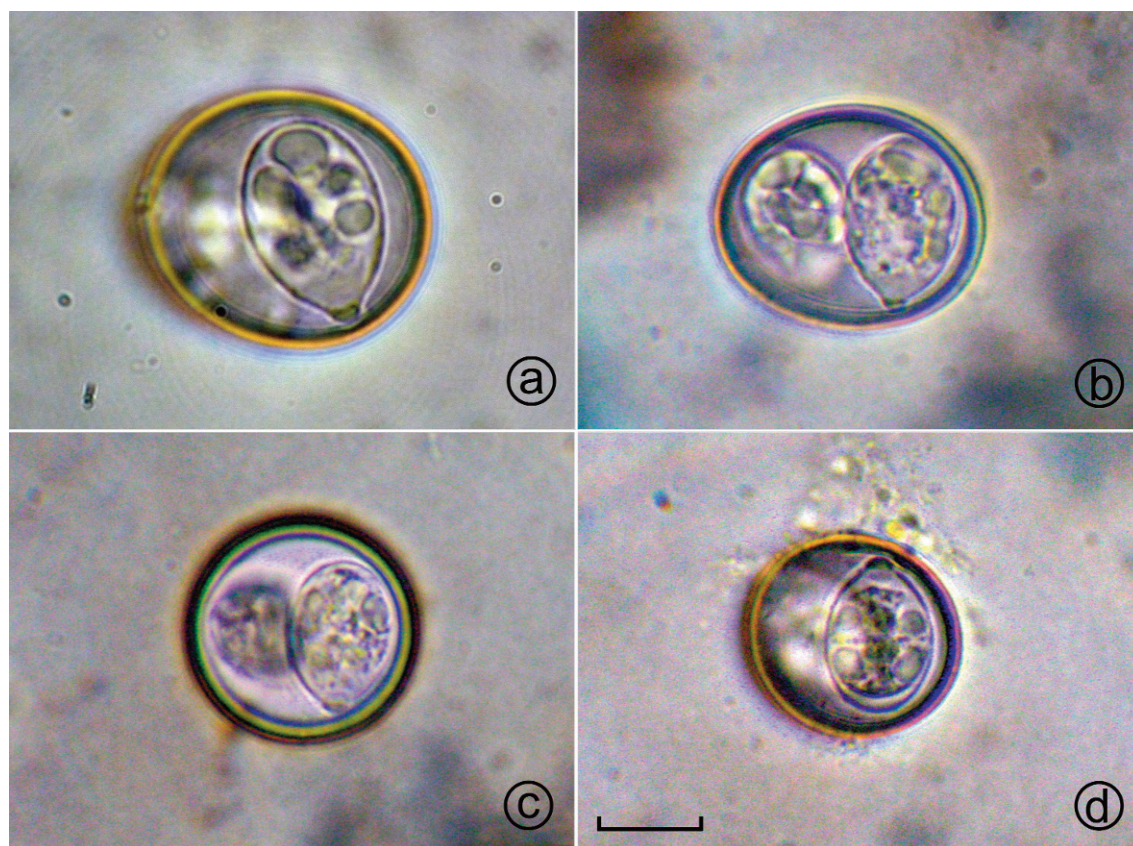


Fig. 3. Photographs of *Isospora cetasiensis* (a, b) and *I. sicalisi* (c, d), new coccidia species recovered from the saffron finch *Sicalis flaveola*. Scale bar = 10 μ m

Type-host: The saffron finch *Sicalis flaveola* Linnaeus, 1766 (Passeriformes, Emberizidae).

Type-locality: Seropédica (22°43'23.79"S and 43°42'36.94"W), Rio de Janeiro, Brazil.

Material deposited: One-half of the oocysts are kept in 10% aqueous buffered formalin (v/v) and the other half in 70% ethanol. 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 are deposited at the same location. The repository number is P-39/2009.

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

Prevalence: 12% (three of 26 examined birds).

Etymology: The specific epithet is derived from the generic name of the type host.

Remarks: The *I. sicalisi* oocysts can be distinguished from *I. cetasiensis* by the size and shape of the SSB. From previous descriptions, *I. sicalisi* presents oocysts with similar dimensions to the oocysts described by McQuistion and Wilson (1988), McQuistion (1990), Ball and Daszak (1997), Berto *et al.* (2009) and Pereira *et al.* (2011). These species: *I. fragmenta* McQuistion et Wilson, 1988; *I. temeraria* McQuistion et Wilson, 1988; *I. daphnensis* McQuistion, 1990; *I. tiaris* Ball

et Daszak, 1997; and *I. frontalis* Berto, Balthazar, Flausino et Lopes, 2009 present PG; *I. teresopolensis* Berto, Balthazar, Flausino et Lopes, 2009 has sporocyst elongated; and *I. bocamontensis* Pereira, Berto, Flausino, Lovato et Lopes, 2011 has SB half-moon-shaped and SR compact. These last characteristics were not found in *I. sicalisi* (Table I).

Discussion

Species of *Isospora* Schneider, 1881 has been described in birds of the family Emberizidae worldwide; however, those coccidia described in the New World assume greater importance due to the higher probability of transmission between sympatric passerines (McQuistion and Wilson 1988, 1989; McQuistion 1990; Carvalho-Filho *et al.* 2005; Berto *et al.* 2009).

The coccidia of this current study were compared in detail with coccidian parasites of New World passerine birds that are feature-similar and belong to the same host family (Duszynski and Wilber 1997, Pereira *et al.* 2011) (Table I). Therefore, these described specimens become the twentieth and twenty-first new coccidian species obtained from the New World emberizid birds.

A few variations in SB and SSB (Figs. 1b-d and 2b-c) were observed in sporocysts of *I. cetasiensis* and *I. sicalisi* similarly to study of Grulet *et al.* (1982); however, these variations are not significant enough to separate and describe them as new species. These variations could be possibly the result of the sporulation process, the position of the SZ inside of the sporocyst, or the position of the oocyst and sporocyst under the coverslip.

The description of new coccidian parasites from emberizid hosts provides significant information, since Brazil has 65 distinct emberizid species catalogued (CBRO 2009). The ease of transmission of isosporoid coccidian and the geographic range of the host, *S. flaveola*, could trigger the dissemination of these new coccidian species to sympatric emberizids in South America.

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