









New *Isoospora* and Host Species in Brazilian Passerines

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■ Keywords

Apicomplexa, *Gnorimopsar chopi*, *Isoospora chopi*, *Isoospora gnorimopsar*, *Paroaria dominicana*, *Isoospora dominicana*, *Saltator similis*, *Isoospora beagai*, *Isoospora ferri*, *Schistocholamys ruficapillus*, *Isoospora ruficapillus*.



ABSTRACT

Normal passerines (n=216) were evaluated for oocysts of *Isoospora* in feces at the Triage Center for Wild Animals (CETAS, IBAMA, Belo Horizonte; August 21 to September 21, 2012). The positive samples with oocysts represented 13.0% of Cardinalidae (n=23), 11.2% of Emberizidae (n=107), 50% of Icteridae (n=10) and 60.3% of Thraupidae (n=68). The probability of fecal oocysts attributable to the host in Thraupidae is higher than in Cardinalidae, Emberizidae, Fringillidae and Turdidae, but similar to Icteridae. No oocysts were found in Fringillidae and Turdidae. Within Thraupidae, *Isoospora* was for the first time described in *Paroaria dominicana* and *Schistocholamys ruficapillus* and within Icteridae, in *Gnorimopsar chopi*. *Saltator similis* presented a higher risk, 66.9% greater than *Lanio pileatus* and *Sporophila caerulescens* and with a 27.9% greater probability than *Sporophila nigricolis*. The new coccidian species described were *Isoospora dominicana* [ellipsoid oocysts, 25 (30-20) x 25 (28-20) µm] in *Paroaria dominicana*; *Isoospora beagai* [ovoid oocysts, 28 (32-17) x 25 (29-16) µm] and *Isoospora ferri* [ellipsoid oocysts, 20 (22-16) x 18 (22-15) µm] in *Saltator similis*; *Isoospora ruficapillus* [spherical to subspherical oocysts, 25 (26-23) x 24 (25-21) µm] in *Schistocholamys ruficapillus*; and *Isoospora chopi* [spherical to sub-spherical oocysts, 24.5 x 22 (30-20 x 25-20) µm] and *Isoospora gnorimopsar* [sub-spherical to ovoid oocysts, 27 x 23 (32-22 x 28-20) µm] in *Gnorimopsar chopi*. The morphometry and features were compared with previously described *Isoospora* in passerines. New coccidian species and new passerine hosts are described for *Isoospora* and recommends for constant monitoring during rehabilitation, especially for the hosts of Thraupidae and Icteridae.

Coccidiosis in the order Passeriformes is caused mostly by the genus *Isoospora* (Page & Haddad, 1995; Duszynski *et al.*, 2018; Freitas *et al.*, 2002; Marietto-Gonçalves *et al.*, 2009; Brown *et al.*, 2010; Costa *et al.*, 2010; Berto *et al.*, 2011a) and may result in important clinical impacts for passerines during captivity (Page and Haddad, 1995; Friend and Frason, 1999; Brown *et al.*, 2010; Berto *et al.*, 2011a). In fact, infection by *Isoospora* represents the most relevant parasitic disease for captive birds (Vilela *et al.*, 2009; Costa *et al.*, 2010; Keeler *et al.*, 2011; Pereira *et al.*, 2011) and demands diagnostic surveillance and strategic or curative medication to reduce losses (Freitas *et al.*, 2002; Yabsley, 2008; Marietto-Gonçalves *et al.*, 2009). In contrast, subclinical infection (coccidiosis) is the most common form of infection in the wild, representing a minor health impact in free-living birds (Upton *et al.*, 1995). However, most species of coccidians, especially of wild birds, are yet to be described (Moore & Clayton, 1997; Lederberg, 1998; Freitas *et al.*, 2002; Wobeser, 2008). In captive birds, a fatal disease outbreak by *Isoospora icterus* was reported in Brazil in a group of a native icterid passerine *Icterus jamacaii* (Campo oriole or Troupial) (Marques *et al.*, 2011).



Twenty-one South-American passerine species are described as hosts for the genus *Isospora*, however, only 19 out of the 1073 species of Brazilian passerines have been studied for the occurrence of *Isospora* or other coccidians (Sick, 1997; Berto *et al.*, 2011a; CBRO, 2014).

The Thraupidae and Icteridae member species are the most frequently sampled among those admitted at the wild animal triage centers in Brazil (Centros de Triagem de Animais Silvestres, CETAS) due to illegal trade (Ferreira & Glock, 2004; Borges *et al.*, 2006; Pagano *et al.*, 2009; Santos *et al.*, 2011; Vilela, 2012; CBRO, 2014) and also because most species are declining in population (IUCN, 2018). According to Vilela (2012), and considering the new classification of species (CBRO, 2014), 61% of all Brazilian passerines at the triage centers belong to Thraupidae. This study describes 5 new host species of *Isospora*, from Thraupidae: (*Paroaria dominicana*, *Schistoclamys ruficapillus* and *Sporophila nigricolis*), of Cardinalidae (*Cyanoloxia brisonii*) and of Icteridae (*Gnorimopsar chopi*), and 2 new species of *Isospora* in the previously known host *Saltator similis*.

We describe the occurrence of oocysts of *Isospora* in passerines of families Thraupidae, Cardinalidae,

Emberizidae, Fringillidae and Turdidae, and evaluate new and previously described host species, with the characterization of oocysts by morphometry and structural features.

MATERIALS AND METHODS

Birds and sampling

Fecal samples (n = 216) of clinically healthy passerines of six taxonomic families were evaluated: Cardinalidae, Emberizidae, Fringillidae, Icteridae, Thraupidae, and Turdidae. The occurrence of coccidia was evaluated for all passerines at rehabilitation in the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012 (Table I).

The study was registered at the SISBIO (Sistema de Autorização e Informação em Biodiversidade (SISBIO) do Instituto Chico Mendes de Conservação da Biodiversidade) at under number 35825-1 and at the ethics committee on animal research (CETEA-UFMG) under number 126/2013.

All fecal samples were collected non-invasively from birds that were kept in individual cages throughout the study. Upon arrival, white paper sheets were left

Table I – Passeriformes evaluated and individuals with oocysts in feces.

Family	Scientific name	Common name	Positive	Negative	Total	% Positive
Cardinalidae			3	20		
	<i>Cyanoloxia brisonii</i>		3	20	23	13.0
Emberizidae			12	95	107	11.2
	<i>Sicalis flaveola</i>	Canário da terra verdadeiro	7	33	40	17.5
	<i>Sporophila angolensis</i>	Curió	0	3	3	0.0
	<i>Sporophila caerulescens</i>	Coleirinho	4	9	13	30.7
	<i>Sporophila frontalis</i>	Pixoxó	0	1	1	0.0
	<i>Sporophila lineola</i>	Bigodinho	0	4	4	0.0
	<i>Sporophila maximiliani</i>	Bicudo	0	3	3	0.0
	<i>Sporophila nigricolis</i>	Baiano	1	34	35	2.8
	<i>Sporophila plumbea</i>	Patativa	0	1	1	0.0
	<i>Zonotrichia capensis</i>	Tico tico	0	7	7	0.0
Fringillidae	<i>Sporagra magellanica</i>	Pintassilgo	0	4	4	0.0
Icteridae			5	5	10	50.0
	<i>Gnorimopsar chopi</i>	Graúna	5	4	9	55.5
	<i>Icterus jamacaii</i>	Corrupião	0	1	1	0.0
Thraupidae			41	27	68	60.3
	<i>Lanio pileatus</i>	Tico tico rei cinza	0	3	3	0.0
	<i>Paroaria dominicana</i>	Cardeal do nordeste	4	3	7	57.1
	<i>Saltator similis</i>	Trinca ferro verdadeiro	36	18	54	33.3
	<i>Saltatriculla atricollis</i>	Bico de pimenta	0	2	2	0.0
	<i>Schistoclamys ruficapillus</i>	Bico de veludo	1	1	2	50.0
Turdidae			0	4	4	0
	<i>Turdus amaurochalinus</i>	Sabiá poca	0	1	1	0.0
	<i>Turdus rufiventris</i>	Sabiá laranja	0	3	3	0.0
Total			61	155	216	28.24

overnight on the bottom of the cages to collect feces the following morning. Samples were immediately examined at microscopy and subjected to flotation in saturated NaCl for microscopy on a slide with coverslip (100, 400 and 1,000x). Oocyst positive samples were partially transferred to an aerated flask with 2.5% potassium dichromate solution (22 C) at 1:5 for sporulation (sporogony) for 1 week. Sporulated oocysts of each bird were subjected to morphologic and morphometric evaluation in a Zeiss Axioscop 40 microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany), equipped with digital imaging, according to the Duszynski & Wilber (1997) protocol. Images were captured using a Canon G10 digital camera (Canon S Tower 2-16-6, Konan MINATO-KU, Tokyo, Japan), a 52mm adaptor tube and Axiovision 4.8 software with a Sony Vaio VPCSB35FB (Sony Brasil, São Paulo, SP, Brazil). Only viable oocysts, showing the complete development of sporocysts and sporozoites were evaluated. The numerical data were evaluated for sampling normality and homogeneity (ANOVA). The morphological features and morphometrical values were evaluated in an entirely random design (ERD) (Mann-Whitney, ANOVA) and the specific correlations were verified for the data of each individual. For *Saltator similis*, data were further evaluated for distribution normality and homogeneity of sampling error (Mann-Whitney, ANOVA) and specific correlation to determine the 2 coccidian species (Sampaio, 2007).

RESULTS

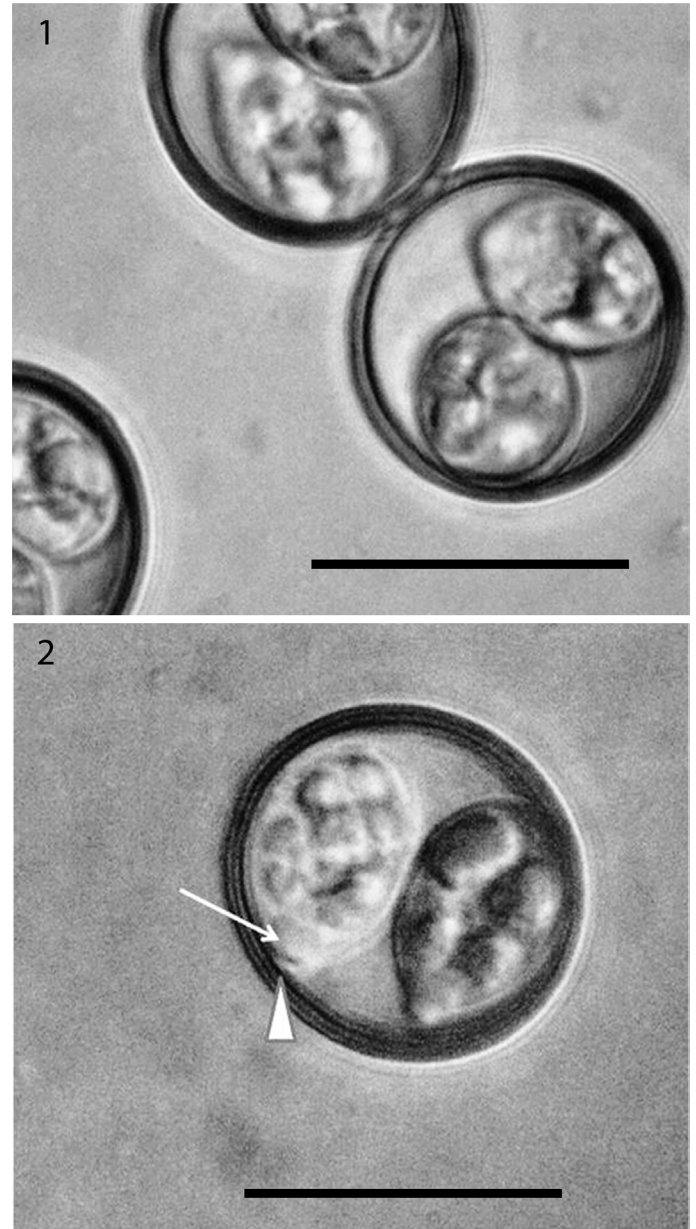
Fecal samples detected positive for oocysts were from *Cyanoloxia brisonii* (Cardinalidae), *Sicalis flaveola*, *Sporophila caerulea* and *Sporophila nigricolis* (Emberizidae), *Gnorimopsar chopi* (Icteridae), and *Schistoclamys ruficapillus*, *Paroaria dominicana*, *Saltator similis*, and *Sporophila nigricolis* (Thraupidae). Samples from Fringillidae and Turdidae were negative (Table I). All sporulated oocysts obtained were typical of the *Isospora* and were further characterized.

DESCRIPTIONS

Isospora dominicana n. sp.

Description of the sporulated oocyst: spherical to subspherical oocysts, 25 x 25 µm (20-28 x 20-30 µm) (n=50); morphometric index (MI, length /width) 1.0 (1.0 – 1.2); smooth bilayered wall (1 µm in thickness); absences of micropyle, polar granule and sporocyst residuum (Table II).

Description of the sporocyst: Ellipsoidal sporocysts, 11 x 17 µm (9-13 x 15-18 µm), SI 1.5 (1.3-1.8), flattened Stieda body (1 µm in height by 2 µm in width) and prominent substieda body (2 µm in height by 3 µm in width) (Table II).



Figures 1, 2 – Oocysts of *Isospora dominicana* recovered from *Paroaria dominicana*. Note the spheric to subspheric oocyst, absence of polar granule and oocyst residuum, flat Stieda body (arrow) and prominent substieda (arrow head).

Taxonomy summary

Host: *Paroaria dominicana* (Aves: Passeriformes: Thraupidae).

Geographical location and date: Samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Undetermined.



Table II – Comparative morphometry (μm) of oocysts and sporocysts, and morphological features of species of genus *Isozona* described in family Thraupidae (Aves: Passeriformes: Thraupidae).

Species	Host	Reference	Oocyst			Sporocyst			Stieda body Shape Length x width (μm)	Residuum	
			Shape	Polar granule	Diameter (μm) Major Minor	Morpho metric index	Shape	Diameter (μm) Major Minor			Morpho metric index
<i>Isozona vaniperorum</i>	<i>Cardinalis cardinalis</i> ; <i>Saltator similis</i>	Levine et al. (1980); Lopes et al. (2007)	Subspherical	Present	23,1 (26-22) Major 22,4 (25-20) Minor	1,04	Ovoid	16,3 (17-15) Major 10 Minor	1,53	Button shaped	Compact
<i>I. paroariae</i>	<i>Paroaria coronata</i>	Upton et al. (1985)	Subspherical	Absent	22,3 (26-19) Major 21,4 (24-18) Minor	1,1	Ovoid	15,2 (17-14) Major 10 Minor	1,57	Mammilliform	Compact
<i>I. pityli</i>	<i>Saltator grossus saturates</i>	McQuiston & Capparella (1992)	Subspherical	Absent	20,1 (21-20) Major 18,8 (20-17) Minor	1,07	Ovoid	14,7 (17-12) Major 9,4 Minor	1,4	Mammilliform	Compact
<i>I. formarum</i>	<i>S. g. grossus</i> ; <i>S. g. saturatus</i>	McQuiston & Capparella (1992)	Subspherical	Absent	24,6 (27-21) Major 23,5 (25-20) Minor	1,1	Ovoid	15,7 (17-14) Major 11,3 Minor	1,5	Mammilliform	Compact
<i>I. saltatori</i>	<i>S. similis</i>	Berto et al. (2008b)	Subspherical	Absent	18,3 (20-17) Major 17,9 (20-16) Minor	1	Ovoid	13,4 (15-12) Major 8,9 Minor	1,5	Mammilliform	Compact
<i>I. trincaferri</i>	<i>S. similis</i>	Berto et al. (2008b)	Subspherical	Present	26,2 (29-24) Major 23,6 (25-22) Minor 19,9 (20-19)	1,1	Ovoid	17,5 (18-17) Major 11,5 Minor	1,5	Bubble shaped	Diffuse
<i>I. thraupis</i>	<i>Thraupis palmarum melanoptera</i>	Lainson (1994); Berto (2010)	Subspherical	Absent	22,6 (21-19) Major 18,7 (20-19) Minor	1,2	Ovoid	14,1 (16-14) Major 8,5 Minor	1,7	Triangular, prominent	Diffuse
<i>I. andesensis</i>	<i>Chlorospingus ophthalmicus</i>	Templar et al. (2004); Berto (2010)	Subspherical or ovoid	Present	22,1 (24-20) Major 18,9 (20-17) Minor	1,2	Ovoid	13,6 (17-9) Major 9 Minor	1,5	Bubble-shaped	Diffuse
<i>I. irisidomisi</i>	<i>Irisidornis analis</i>	Metzlaaris et al. (2005); Berto (2010)	Ovoid	Present	25,2 (25-20) Major 23,4 (23-16) Minor	1,03	Ovoid	17,7 (19-17) Major 11,5 Minor	1,54	Flat	Diffuse
<i>I. tiesanguii</i>	<i>Ramphocelus bresilius dorsalis</i> ; <i>T. palmarum</i> ; <i>Dacnis cayana</i>	Berto et al. (2008a); Berto (2010)	Subspherical	Absent	24,2 (26-22) Major 21,9 (26-21) Minor	1	Ellipsoid	22,6 (24-21) Major 13 Minor	1,5	Flat	Diffuse
<i>I. marambaensis</i>	<i>R. b. dorsalis</i>	Berto et al. (2008a, 2010)	Subspherical	Absent	25,5 (31-27) Major 23,8 (29-26) Minor	1,1	Ellipsoid	16,9 (18-16) Major 11 Minor	1,5	Mammilliform	Diffuse
<i>I. sepebibensis</i>	<i>R. b. dorsalis</i>	Berto et al. (2008a, 2010)	Subspherical	Present	21,4 (29-24) Major 20,6 (26-22) Minor	1,1	Ellipsoid	16,1 (19-14) Major 10,2 Minor	1,6	Flat	Diffuse
<i>I. cadimi</i>	<i>R. b. dorsalis</i>	Berto et al. (2009a, 2010)	Subspherical	Absent	23,7 (26-22) Major 22,8 (24-21) Minor	1,04	Ellipsoid or ovoid	16 (18-14) Major 9,9 Minor	1,41	Button-shaped	Diffuse
<i>I. navarroi</i>	<i>R. b. dorsalis</i>	Berto et al. (2009b, 2010)	Subspherical	Absent	22,1 (24-19) Major 21 (23-17) Minor	1	Ovoid	17 (19-15) Major 10,5 Minor	1,7	Mammilliform	Diffuse
<i>I. ramphoceli</i>	<i>Thraupis sayaca</i>	Berto et al. (2009c, 2010)	Subspherical	Absent	25,5 (28-22) Major 22,6 (25-19) Minor	1,1	Piriform	17,6 (18-17) Major 10,5 Minor	1,7	Delicate	Compact
<i>I. silvasouzai</i>	<i>T. sayaca</i>	Berto et al. (2009c, 2010)	Subspherical	Present	28,9 (30-28) Major 27,4 (29-24) Minor	1,1	Bottle-shaped	23,4 (25-23) Major 11,8 Minor	2	Prominent	Diffuse
<i>I. sayacae</i>	<i>T. sayaca</i>	Berto et al. (2009c, 2010)	Spheric to subspherical	Present	27,5 (29-26) Major 25,9 (28-24) Minor	1,1	Ellipsoid or ovoid	17,4 (19-15) Major 12,2 Minor	1,4	Button-shaped	Diffuse
<i>Isozona similis</i>	<i>S. similis</i>	Coelho et al. (2013)	Spheric to subspherical	Present	25 (30-20) Major 25 (28-20) Minor	1,04 (1,18-1)	Ellipsoid	17 (18-15) Major 11 Minor	1,5 (1,8-1,3)	Mammilliform	Absent
<i>Isozona dominicana</i>	<i>Paroaria dominicana</i>	Present study	Spheric to subspherical	Absent	28 (32-17) Major 25 (29-16) Minor	1,15 (1,5-1)	Ovoid	18 (23-12) Major 11 (15-9) Minor	1,53 (2,2-1,23)	Prominent, Button-shaped	Compact
<i>Isozona beagai</i>	<i>Saltator similis</i>	Present study	Spheric to subspherical	Present	20 (22-16) Major 18 (22-15) Minor	1 (1,15-1)	Ellipsoid	14 (20-12) Major 8 Minor	1,6 (2,5-1,3)	Flat	Diffuse
<i>Isozona ferri</i>	<i>Saltator similis</i>	Present study	Spheric to subspherical	Present	25 (26-23) Major 24 (25-21) Minor	1,04 (1,09-1)	Ellipsoid	17 (18-16) Major 11 (11-10) Minor	1,6 (1,63-1,45)	mammilliform	Diffuse

Sporulation: Exogenous.

Frequency of detection: In 4/7 (57%).

Collection material Id. Dominicana: Refrigerated (4 - 8 °C) for analysis and subsequently frozen feces; oocysts photomicrographs deposited at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name given to this species was based on its host name: *Paroaria dominicana*.

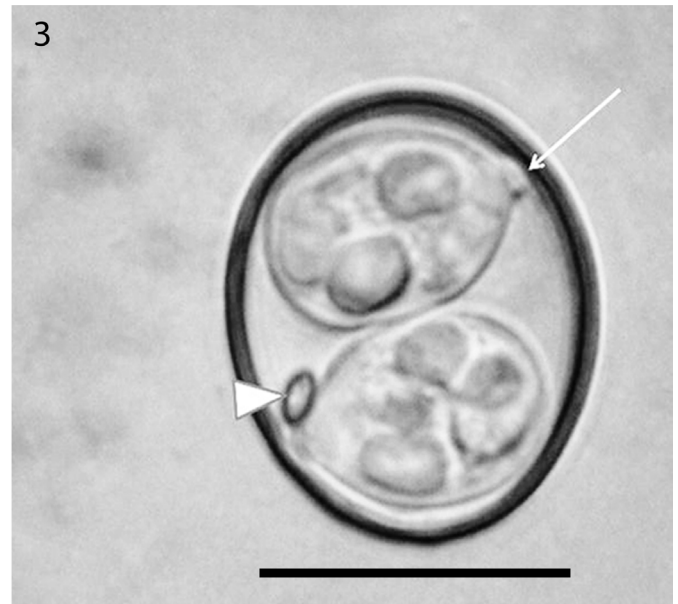
Remarks

Considering the absence of a polar granule found in the coccidian species, this species was similar to 12 others previously described in publications on Thraupidae *Iso*spora *marambaiensis*, *Iso*spora *sepetibensis* and *Iso*spora *tiesangui*, Berto *et al.* (2008a) and Berto (2010), *Iso*spora *saltatori* Berto *et al.* (2008b), *Iso*spora *cadimi* and *Iso*spora *navarroii* Berto *et al.* (2009a, 2009b), *Iso*spora *ramphoceli*, *Iso*spora *sanhaci* and *Iso*spora *sayacae* Berto *et al.* (2009c, 2010), *Iso*spora *thraupis* Lainson (1994), *Iso*spora *formarum* and *Iso*spora *pityli* McQuiston and Capparella (1992), and *Iso*spora *paroariae* Upton *et al.* (1985). Regarding the sporocyst residuum, it was absent for only the described species, in contrast to those compared with *Iso*spora of Thraupidae (Table II). Despite such detail being considered sufficient for describing a new species (Duszynski & Wilber, 1997), further data were analyzed using a dichotomic key (Berto *et al.*, 2010). Results indicated a new species characterized by the absence of polar granule, larger than 25µm in length, flat Stieda body, large and easily visible substieda body and no sporocyst residuum (Table II). The absence of sporocyst residuum was not previously described for *Iso*spora of Passeriformes. Considering these exclusive characteristics, the new species was thereby named *Iso*spora *dominicana*.

*Iso*spora *beagai* n. sp.

Description of the sporulated oocyst: Ovoidal oocysts, 25 x 28µm (16 -29 x 17-32µm) (n = 53), with morphometric index (MI, length/width) 1.1 (1..0 – 1.5), smooth bilayer wall approximately 1 µm in thickness. Absence of micropyle and the presence of polar granule (Table II).

Description of the sporocyst: The sporocysts are ovoidal of 11 x 18 µm (9-15 x 12 - 23 µm), SI 1.5 (1.2 – 2.2), prominent club-shaped Stieda body, of 2 µm in height by 3 µm in width and compact sporocysts residuum (Table II).



Taxonomy summary

Host: *Saltator similis* (Aves: Passeriformes: Thraupidae).

Geographical location and date: Samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Undetermined. Samples were taken from feces.

Sporulation: Exogenous.

Frequency of detection: In 36/54 (68%).

Collection material Id. Beagai: Refrigerated (4-8 °C) for analysis and subsequently frozen feces; oocysts



photomicrographs; stored at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name given to this species was based on the acronym of the geographical location in which it was found (BH, Belo Horizonte) (Brazil).

Remarks

Comparing the oocysts possessing a polar granule with previously described species (Table II), it was shown that, among the 23 known species, including the 4 described in this manuscript, only 8 *Isoospora* species have been characterized by the presence of a polar granule. The species described here (Table III) (*Isoospora beagai*) had a bubble-shaped polar granule similar to *Isoospora vanriperorum*, *Isoospora trincaferri*, *Isoospora iridornisi*, *Isoospora sepetibensis* and *Isoospora similis*. Considering the sporocyst residuum, a compact residuum similar to *I. vanriperorum* and *I. sepetibensis* was observed. In order to compare the species described here to the previously described *I. vanriperorum*, data in Table II along with descriptive images previously published, were employed (Lopes *et al.*, 2007; Berto *et al.*, 2011a; 2011b). Dissimilarities were found when compared to *I. vanriperorum*, such as a bilayered wall and a large and conspicuous substieda body. The sporocyst, although both ovoidal, also showed differences. Such characteristics are considered sufficient in determining this to be a different species (Duszynski & Wilber, 1997).

When considering *I. sepetibensis*, comparisons using dichotomic keys, descriptive drawings (Berto *et al.*, 2010; 2011a; 2011b), and data in Table II, show that sporocysts were described as ellipsoidal, in contrast to the ovoidal sporocysts found in the new *I. beagai* species found in this paper. The sporocyst residuum has been reported as compact, in contrast to the slightly larger and diffused sporocyst described here. Comparing the morphometric data of *I. beagai* with that of *I. sepetibensis*, a slight difference of oocyst and sporocyst measurements were revealed, being greater for the former.

The dichotomic key (Berto *et al.*, 2010) suggests this species be compared to *I. iridornisi*, which presents larger oocysts and sporocysts than *Isoospora beagai* ($p = 0.0001$), a compact but not diffused sporocyst residuum in *I. beagai*, a larger substieda body, and not collar-shaped as seen in *I. beagai*. These individual features were considered sufficient in order to describe a new species (Duszynski & Wilber, 1997). In addition, the described host (*Irisidornis analis*) for *Isoospora iridornisi* occurs only in Colombia, Ecuador and Peru (northern South America), while *Saltator similis* occurs

Table III – Morphometric comparisons of *Isoospora* species described in passerines of family Icteridae (Aves: Passeriformes, Icteridae).

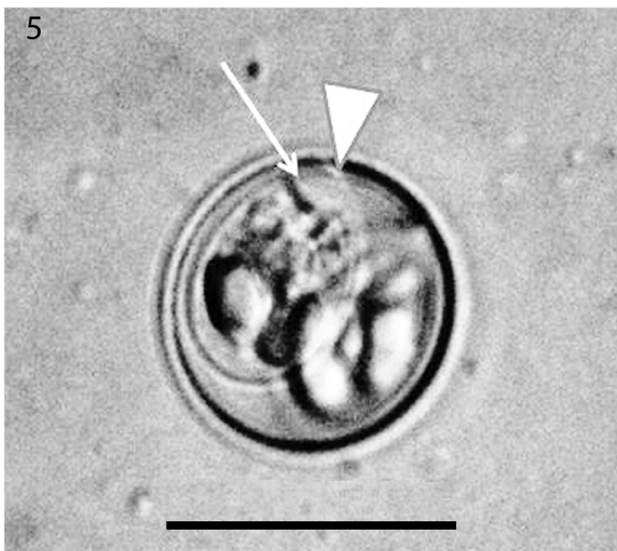
Species	Host	Reference	Oocyst				Sporocyst				
			Shape	Polar granule	Diameter (µm) Length (L) Width (W)	Morphometric Index	Shape	Length (L) Width (W)	Morphometric index	Stieda body Shape height x width (µm)	Residuum
<i>Isoospora divitis</i>	<i>Dives atrovioleaceus</i>	Pellérdy (1967)	Sub-spheric	Absent	(30-22) 28,20	1,1	17	13	1,3	Conspicuous	Diffuse
<i>Isoospora cacici</i>	<i>Cacicus cela</i>	Lainson (1994)	Sub-spheric	Present 1 or 2	26,5 (27,5-22,5) (26,2-20)	1,1	17,7 (18,75-17,5)	12,5 (13,75-11,25)	1,4	Prominent, cork shaped	Compact
<i>Isoospora bellicosa</i>	<i>Sturnella bellicosa</i>	Upton et al. (1995)	Episoidal or ovoid	Present 1 or 2	29,4 (26,4-23,5) (26,4-21)	1,2	17,8 (18,6-16,8)	10,7 (11,2-10,2)	1,67	Large	Diffuse
<i>Isoospora icterus</i>	<i>Icterus icterus</i>	Upton & Whitaker (2000)	Sub-spheric	Present	28,9 (32-27,2) (30-25)	1,1	17,8 (19,2-16,8)	12,8 (13,6-12)	1,39	Small, mammiliform	Diffuse
<i>Isoospora icterus</i>	<i>Icterus jamaicai</i>	Marques et al. 2011	Sub-spheric	Present	30,1 (32,5-27,5) (30-26,25)	1,1	17,6 (20-15)	12,9 (15-12,5)	1,4	Small, mammiliform	Diffuse
<i>Isoospora graceannae</i>	<i>Icterus graceannae</i>	Upton & Whitaker (2000)	Sub-spheric	Present	23,9 (26,4-20) (25-18,6)	1,1	15,5 (16,2-14,2)	10,7 (11,4-10)	1,44	Robust	Diffuse
<i>Isoospora gnorimopsar chopi</i>	<i>Gnorimopsar chopi</i>	Present study	Sub-spheric to ovoid	Present 1 or 2	27 (32-22) (28-20)	1,18 (1,33-1)	16 (19-14)	11 (12-9)	1,52 (1,72-1,33)	Flat 1 x 2	Absent
<i>Isoospora chopi</i>	<i>Gnorimopsar chopi</i>	Present study	Sub-spheric to ovoid	Absent	24,5 (30-21) (25-20)	1,08 (1,19-1)	15 (16-13)	9,7 (10-8)	1,57 (1,66-1,3)	Prominent 2 x 1	Present

in southcentral South America, including Argentina, Bolivia, Brazil, Paraguay and Uruguay.

The morphologic and morphometric data and images were compared with descriptions in literature (Table II). The differences described for *I. beagai*, in comparison to *I. iridornisi*, include a diffused and ovoidal sporocyst for *I. beagai*, in contrast with a compact and ellipsoidal sporocyst for *I. iridornisi*.

Considering the morphometrical data of oocysts in *Saltator similis* (Tables II and III), two different set of data were obtained, and oocyst dimensions, the presence of polar granule, and compact sporocyst residuum, were considered sufficient reasons to describe a new species *Iso*spora *beagai* (Duszynski & Wilber, 1997).

***Iso*spora *ferri* n.sp.**



Figures 5, 6 – Oocysts of *Iso*spora *ferri* recovered from *Saltator similis*. (5) Note the spheric to subspheric oocyst, absence of polar granule, flat delicate Stieda body (arrowhead), large and conspicuous substieda (arrow), and round oocyst. (6) Note the absence of polar granule, flat and delicate Stieda body (arrowhead), and diffused sporocyst residuum (arrows).

Description of the sporulated oocyst: Spherical to subspherical oocysts, 18 x 20 μ m (15-22 x 16-22) (n=59), MI 1.0 (1.0 x 1.2), presenting a smooth double layered wall of approximately 1 μ m, and the absence of micropyle, polar granule and diffused sporocyst residuum (Table II).

Description of the sporocyst: Sporocysts are ellipsoidal, 8 x 14 μ m (7 – 11 x 12 – 20) SI, 1,6 (1.3 – 2.5), with flat Stieda body 1 μ m in height and 2 μ m in width, prominent substieda body of approximately 2 μ m in height and 3 μ m in width (Table II).

Taxonomy summary

Host: *Saltator similis* (Aves: Passeriformes: Thraupidae).

Geographical location and date: samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Undetermined. Samples were taken from feces.

Sporulation: Exogenous.

Frequency of detection: In 36/54 (68%).

Collection material Id. Ferri: Refrigerated (4-8 °C) for analysis and subsequently frozen feces; oocysts photomicrographs; stored at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name *I. ferri* was given based on the host's popular Portuguese name: trinca-ferro.

Remarks

In comparison to the coccidia previously found in feces of *S. similis* (Table II), the oocysts in this study were larger, thus reinforcing the need to introduce a separate species (Duszynski & Wilber, 1997).

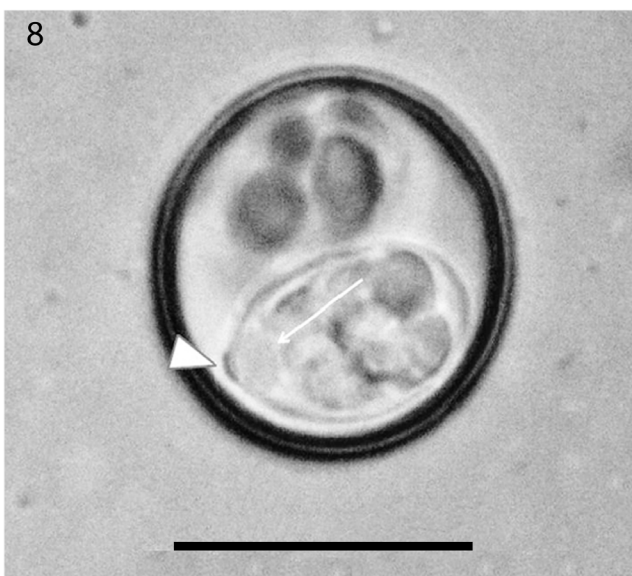
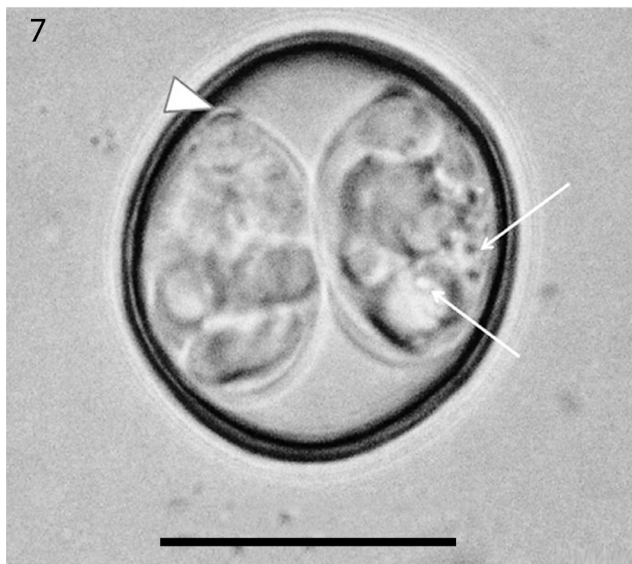
Oocysts of *Iso*spora, which do not present polar granule of previously described species in *S. similis*, were compared to those of this new species, *I. ferri* (Table II). Out of the 20 known species, in addition to 4 described in this family (Thraupidae), 16 do not present a polar granule oocyst. The dichotomic key (Berto *et al.*, 2010) suggests this species be compared to *Iso*spora *tiesanguis* of the Brazilian tanager *Ramphocelus bresilius*. However, comparing the oocyst and sporocyst of these coccidians, *I. ferri* had a smaller dimension (Table II). The oocyst format was ovoidal for *I. ferri* but ellipsoidal for *I. tiesanguis*; the Stieda body measured 0.5 x 3 μ m in *I. tiesanguis* in comparison to 1 x 2 μ m in *I. ferri*; and the substieda body measured 2.5 x 4 μ m in *I. tiesanguis*, but 2 x 3 μ m for *I. ferri*. Such differences are considered sufficient in determining

this to be a different species of *Isospora* in *Saltator similis* (Duszynski & Wilber, 1997).

The differences to *Isospora navarroi* were visible at the Stieda and substieda bodies (Berto *et al.*, 2011a, 2011b). The new species presented a larger flat Stieda and larger conspicuous substieda body. The sporocyst residuum was diffused and spread within the sporocyst, and not ring-shaped as in *I. navarroi*.

Considering the morphometrical data of oocysts in *Saltator similis* (Tables II and III), two different sets of data were obtained, and oocyst dimensions, the absence of polar granule, and diffuse sporocyst residuum, considered sufficient reasons enough to indicate *Isospora ferri* as a new species.

Isospora ruficapillus



Figures 7, 8 – Oocysts of *Isospora ruficapillus* recovered from *Schistoclamys ruficapillus*. (7) Note the mammilliform Stieda body (arrowhead) and the sporocyst diffused residuum (arrow). (8) Note the complex formed by the mammilliform Stieda body (arrowhead) and the prominent substieda (arrow).

Description of the sporulated oocyst: Spherical to subspherical oocysts, 24 x 25 μm (21-25 x 23-26) (n = 20), MI, 1.0 (1.0 – 1.1), presenting a double smooth wall, approximately 1 μm in thickness, the absence of micropyle and polar granule (Table II).

Description of the sporocyst: The ellipsoidal sporocysts, 11 x 17 μm (10 -11 x 16 x 18), with mammilliform Stieda body 1 μm in height per 2 μm in width, prominent substieda 2.5 x 3 μm (2.5 μm in height by 3 μm in width), and the presence of a diffused sporocyst residuum (Table II).

Taxonomic summary

Host: *Schistoclamys ruficapillus* (Aves: Passeriformes: Thraupidae).

Geographical location and date: Samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Undetermined. Samples were taken from feces.

Sporulation: Exogenous.

Frequency of detection: 1/2 (50%).

Collection material Id. *Ruficapillus*: Refrigerated (4-8 °C) for analysis and subsequently frozen feces; oocysts photomicrographs stored at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name *I. Ruficapillus* was given based on the species' host *Schistoclamys ruficapillus*.

Remarks

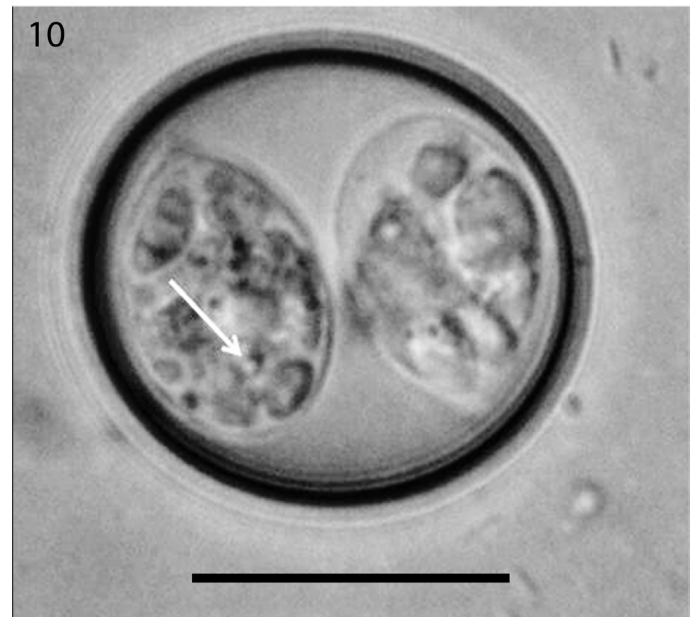
The morphometric and morphologic data obtained after sporulation were compared to data from the literature (Table II) and using a previously described dichotomic key (Berto *et al.*, 2010) which enabled the description of a new species: *Isospora ruficapillus*.

The closest species to *Isospora ruficapillus* was *Isospora sanhaci*, based on the dichotomic key (Berto *et al.*, 2010). *I. sanhaci* was described as having a roundish delicate substieda body, which differed from *I. ruficapillus* as observed in the present study, even though it presented similarities in other structures. The sporocyst shape of both species differed, being ellipsoidal for *I. ruficapillus* and ovoid for *I. sanhaci*. Considering the sporocyst residuum, the new described species presented a diffused structure.

Taking into account the absence of polar granule, the shape of the Stieda body and sporocyst residuum; the species found in *Schistoclamys ruficapillus* suggests this species be compared to *Isospora navarroi* and *Isospora marambaiensis* previously

described in *Ramphocelus bresilius dorsalis* (Berto *et al.*, 2008a; 2009b; 2010). *I. marambaiensis* presented larger oocysts and sporocysts, but the Stieda and substieda bodies were smaller and more delicate than in *I. ruficapillus* (Berto *et al.* 2011a, 2011b), and such differences were considered sufficient for describing a new coccidian species (Duszynski & Wilber, 1997).

Isoospora chopi



Figures 9, 10 – Oocysts of *Isoospora chopi* recovered from *Gnorimopsar chopi*. (9) Note the spheric to subspheric oocyst of *Isoospora chopi* without polar granule, with prominent Stieda body (arrow) and diffused sporocyst residuum (arrowhead). (10) Note the spheric to subspheric *Isoospora chopi* oocyst with sporocyst residuum (arrow), prominent Stieda body (arrowhead) and absence of polar granule.

Description of the sporulated oocyst: Spheric to subspheric oocysts (n = 48) of 22 x 24.5µm (20-25 x 20-30µm), MI of 1.2 (1.0 -1.2), with smooth double layered wall of approximately 1 µm in thickness, without micropyle or polar granule (Table IV).

Table IV – Morphometric comparisons of *Isoospora* found in feces* of *Gnorimopsar chopi* (Aves, Passeriformes: Icteridae) in triage in the Wild Animals Triage Center (CETAS) of Belo Horizonte, Minas Gerais, Brazil.

Species	Mean	Standard deviation	Median	Maximum	Minimum	Prob. (P=)
Oocyst length						
1	24,23	1,94	24,5	30,00	20,00	0,0001
2	26,95	2,32	27,00	32,00	22,00	
Oocyst width						
1	22,52	1,57	22,00	25,00	20,00	0,5635
2	22,81	1,84	23,00	28,00	20,00	
Oocyst morphometric index						
1	1,08	0,06	1,08	1,20	1,00	0,0001
2	1,19	0,11	1,18	1,52	1,00	
Sporocyst length						
1	15,05	0,95	15,00	16,00	13,00	0,0001
2	16,33	1,03	16,00	19,00	14,00	
Sporocyst width						
1	9,70	0,57	10,00	10,00	8,00	0,0001
2	10,69	0,64	11,00	12,00	9,00	
Sporocyst morphometric index						
1	1,55	0,08	1,58	1,67	1,30	0,2028
2	1,53	0,10	1,52	1,73	1,33	

*Fecal samples were collected from August 21 to September 21, 2012.

Species 1: proposed name *Isoospora chopi*; species 2: proposed name *Isoospora gnorimopsar*.

Description of sporocyst: Ellipsoidal sporocysts of 10 x 15 µm (8-10 x 13-16 µm) with 2 µm in height and 1 µm in width prominent Stieda body and diffused sporocyst residuums (Table IV)

Host: *Gnorimopsar chopi* (Aves: Passeriformes: Icteridae).

Geographical location and date: Samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Unknown. Samples were taken from feces.

Sporulation: Exogenous. **Frequency of detection:** In 5/9 (55%).

Collection material Id. Chopi: Refrigerated (4-8 °C) for analysis and subsequently frozen feces; oocysts photomicrographs stored at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name given to this species was based on its host name: *Gnorimopsar chopi*.

Remarks

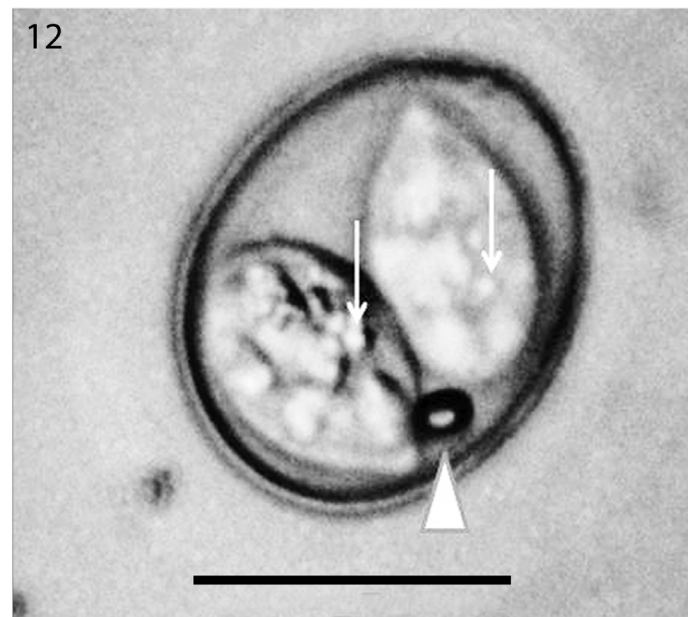
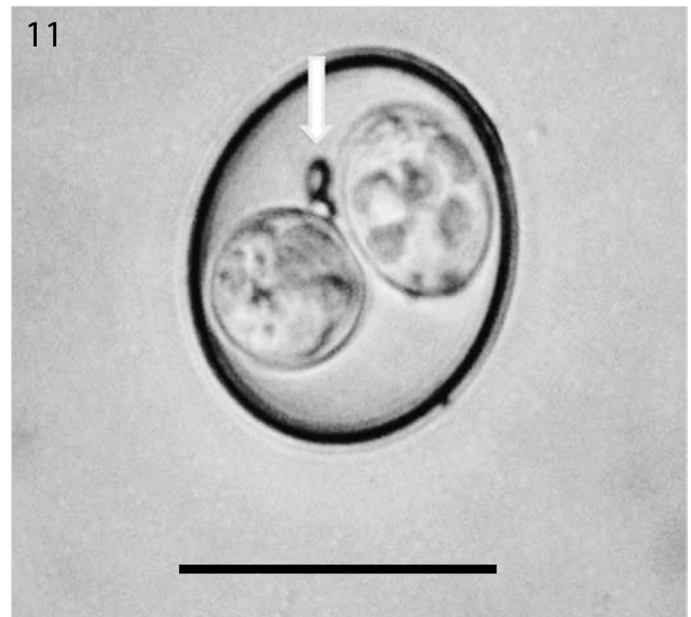
The morphometric and morphologic data (Table IV) obtained after sporulation were compared to data from the literature (Table V) for *Isospora* of Icteridae and using a previously described dichotomic key (Berto *et al.*, 2010) which enabled the description of a new species, *Isospora chopi*.

Analyzing the previously known *Isospora* of Icteridae, only *Isospora divitis* (Pellérdy, 1967) (Table V) shares common features to the new species (*I. chopi*), including the presence of sporocyst residuum, even though the morphometric measurements of the sporocyst differed. *I. divitis* was described in *Dives atrovioleaceus* as an endemic icterid in Cuba. *D. atrovioleaceus* and *G. chopi* never share the same environment; *G. chopi* occurs up to the southern borders of the Amazon forest in Brazil and *D. atrovioleaceus* occurs only in Cuba.

Isospora gnorimopsar

Description of the sporulated oocyst: Subspheric to ovoid oocysts of 23 - 27 µm (20-28 x 22-32µm) (n = 59), with morphometric index (MI) 1.2 (1.0-1.4), with a smooth double layered wall of approximately 1 µm in thickness, absent micropyle and with 1 or 2 polar granules (Table IV).

Description of the sporocyst: Ellipsoidal sporocysts, 11-16 µm (9-12 x 14-19 µm), with SI 1.5 (1.3 x 1.), flat Stieda body (1 µm in thickness and 2 µm in width) and diffused sporocyst residuum (Table IV).



Figures 11, 12 – Oocysts of *Isospora gnorimopsar* recovered from *Gnorimopsar chopi*. (11) Note the ovoid to subspheric oocyst of *Isospora gnorimopsar* with two polar granules (arrow). (12) Note the ovoidal to subspheric oocyst of *Isospora gnorimopsar* with one polar granule (arrowhead) and sporocyst residuum (arrows).

Taxonomic summary

Host: *Gnorimopsar chopi* (Aves: Passeriformes: Icteridae).

Geographical location and date: Samples obtained at the Wild Animal Triage Center of Belo Horizonte (CETAS/BH), Brazil, from August 21st to September 21st, 2012.

Site of infection: Unknown. Samples were taken from feces.

Sporulation: Exogenous.

Frequency of detection: In 5/9 (55%).



Table V – Morphometric comparisons of *Isoospora* species described in passerines of family Icteridae (Aves: Passeriformes, Icteridae).

Species	Host	Reference	Oocyst			Sporocyst			Stieda body	Residuum		
			Shape	Polar granule	Diameter (µm)	Morphometric Index	Shape	Length (L)			Width (W)	Morphometric index
<i>Isoospora divitis</i>	<i>Dives atroviolaceus</i>	Pellérdy (1967)	Sub-spheric	Absent	(30-22)	(28-20)	1,1	17	13	1,3	Conspicuous	Diffuse
<i>Isoospora cacici</i>	<i>Cacicus cela</i>	Lainson (1994)	Sub-spheric	Present 1 or 2	26,5 (27,5-22,5)	23,7 (26,2-20)	1,1	17,7 (18,75-17,5)	12,5 (13,75-11,25)	1,4	Prominent, cork shaped	Compact
<i>Isoospora bellicosa</i>	<i>Sturnella bellicosa</i>	Upton et al. (1995)	Episoidal or ovoid	Present 1 or 2	29,4 (26,4-23,5)	23,5 (26,4-21)	1,2	17,8 (18,6-16,8)	10,7 (11,2-10,2)	1,67	Large	Diffuse
<i>Isoospora icterus</i>	<i>Icterus icterus</i>	Upton & Whitaker (2000)	Sub-spheric	Present	28,9 (32-27,2)	27,2 (30-25)	1,1	17,8 (19,2-16,8)	12,8 (13,6-12)	1,39	Small, mammiliform	Diffuse
<i>Isoospora icterus</i>	<i>Icterus jamaicai</i>	Marques et al. 2011	Sub-spheric	Present	30,1 (32,5-27,5)	28,5 (30-26,25)	1,1	17,6 (20-15)	12,9 (15-12,5)	1,4	Small, mammiliform	Diffuse
<i>Isoospora graceannae</i>	<i>Icterus graceannae</i>	Upton & Whitaker (2000)	Sub-spheric	Present	23,9 (26,4-20)	22,3 (25-18,6)	1,1	15,5 (16,2-14,2)	10,7 (11,4-10)	1,44	Robust	Diffuse
<i>Isoospora gnorimopsar</i>	<i>Gnorimopsar chopi</i>	Present study	Sub-spheric to ovoid	Present 1 or 2	27 (32-22)	23 (28-20)	1,18 (1,33 - 1)	16 (19-14)	11 (12 - 9)	1,52 (1,72 - 1,33)	Flat 1 x 2	Absent
<i>Isoospora gnorimopsar</i>	<i>Gnorimopsar chopi</i>	Present study	Sub-spheric to ovoid	Absent	24,5 (30-21)	22 (25-20)	1,08 (1,19 - 1)	15 (16-13)	9,7 (10-8)	1,57 (1,66-1,3)	Prominent 2 x 1	Present

Collection material Id. *Gnorimopsar*: Refrigerated (4-8 °C) for analysis and subsequently frozen feces; oocysts photomicrographs stored at the Avian Diseases Laboratory, Veterinary College, UFMG.

Etymology: The name given to this species was based on the genus of the host, *Gnorimopsar chopi*.

Remarks

The morphometric and morphologic data obtained after sporulation were compared to data from literature (Table II) and using a previously described dichotomic key (Berto *et al.*, 2010) which enabled the description of a new species, *Isoospora gnorimopsar*.

The presence of the polar granule in *I. gnorimopsar*, were compared to those of *I. cacici* (Lainson, 1994), *I. bellicosa* (Upton *et al.*, 1995), *I. icterus* (Upton and Whitaker, 2000) and *I. graceannae* (Upton and Whitaker, 2000). However, the flattened shape of the Stieda body was distinct in *I. gnorimopsar*. Although *I. icterus* was described with a small Stieda body, the oocyst and sporocyst average dimensions were significantly different. In addition, no sporocyst residuum was found in *I. gnorimopsar*, in contrast to a diffused residuum in *I. icterus*. The analyses were performed based on descriptions by Berto *et al.* (2011a). Such differences are considered sufficient in determining this to be a new coccidian species (Duszynski & Wilber, 1997).

DISCUSSION

Out of the 216 fecal samples evaluated, 61 were positive for oocysts, which corresponded to 28% of this total, all being of the genus *Isoospora*. Considering this occurrence, similar results had been previously found in birds by McQuiston (2000), Marietto-Gonçalves *et al.* (2009), Vilela *et al.* (2009) and Schoener *et al.* (2013). Considering passerines (Passeriformes), results are in agreement with previous reports by Pereira *et al.*, (2011) and Coelho *et al.* (2013), studying *Gubernatrix cristata* and *Saltator similis*, respectively, and found only *Isoospora*.

Although the impact of coccidiosis in free-living passerines is unknown, the disease is highly significant in captive birds (Swayne *et al.*, 1991; Cork and Alley, 1999; Twentyman, 2001; Gill and Paperna, 2008; Marques *et al.*, 2011; Vasconcelos *et al.*, 2012). Results indicate that the triage birds evaluated, despite being temporarily in captivity, have undergone challenge, which could eventually have become clinically significant for susceptible birds, with the increasing concentration of oocysts in the housing environment. The adequate



cleaning and disinfection of the premises should be intensified using more efficient principles such as ammonium hydroxide (Kahrs, 1995). Considering the risk of coccidiosis for birds in triage, in agreement with monitoring recommendations, the authors suggest routine evaluations of feces to be performed weekly (Friend and Franson, 1999).

In this study, not all oocysts found were characterized. A few samples, although adequate for determining the genus, were not adequate for the description of species due to the lack of development, presentation or preservation of discernible features. New host species presenting *Isoospora* in this study include *Cyanoloxia brisonii*, *Gnorimopsar chopi*, *Paroaria dominicana*, *Schistochlamys ruficapillus*, *Sicalis flaveola*, *Sporophila caerulea* and *Sporophila nigricolis*. The coccidians found in the host species *Cyanoloxia brisonii*, *Sicalis flaveola*, *Sporophila caerulea* and *Sporophila nigricolis* belong to the genus *Isoospora*, and a detailed morphology of the oocysts was not evaluated.

The morphological comparisons were performed for characterizing *Isoospora* species in the host family taxon. The careful analysis of oocysts included the determination of oocyst and sporocyst metrics (length and width), oocyst wall, including layers, projections, texture and thickness, and the presence and characteristics of structures (micropyle, polar granule, oocyst residuum, Stieda and substieda bodies, refractile bodies, nucleus, residuum, adherent membranes, sporodinium and sutures) (Duszynski & Wilber, 1997). When comparing features, *Isoospora gnorimopsar* differed from *Isoospora cacici* (Lainson 1994), *Isoospora belicosa* (Upton *et al.*, 1995), *Isoospora icterus* and *Isoospora graceannae* (Upton and Whitaker 2000). *Isoospora chopi* was compared to *Isoospora divitis* (Pellérdy 1967) and differed from this species.

Isoospora dominicana, *I. beagai*, *I. ferri* and *I. ruficapillus*, which were found in the Thraupidae feces, were compared to and differed from *I. vanriperorum* (Levine *et al.*, 1980; Lopes *et al.*, 2007), *I. paroariae* (Upton *et al.*, 1985), *I. pityli* and *I. formarum* (McQuiston and Capparella, 1992), *I. saltatori* (Berto *et al.* 2008b), *I. trincaferri*, (Berto *et al.*, 2008b), *I. thraupis* (Lainson, 1994; Berto, 2010), *I. andesensis* (Templar *et al.*, 2004; Berto, 2010), *I. irisidornisi* (Metzelaars *et al.*, 2005; Berto, 2010), *I. tiesangui* (Berto *et al.*, 2008a; Berto, 2010), *I. marambaiensis* (Berto *et al.* 2008a; 2010), *I. sepetibensis* (Berto *et al.*, 2008a; 2010), *I. cadimi* (Berto *et al.*, 2009a; 2010), *I. navarroi* (Berto *et al.*, 2009b; 2010), *I. ramphoceli* (Berto *et al.*, 2010), *I. sanhaci* (Berto *et al.*, 2009c; 2010), *I. silvasouzai* (Berto

et al., 2009c; 2010), *I. sayacae* (Berto *et al.*, 2009c; 2010) and *I. similis* (Coelho *et al.*, 2013).

The morphometric and morphologic comparisons enabled the identification of new *Isoospora* species in previously known and unknown passerine host species. However, more reliable and conclusive studies should be developed, including phylogenetic studies, in order to evaluate, compare and group genotypes, using molecular techniques.

In addition to previous studies, we have demonstrated the occurrence of new host species and new coccidian species in native avian host passerine species. The higher risk for the host species of Thraupidae became clear, as compared to Cardinalidae, Emberizidae, Fringillidae and Turdidae. Within Thraupidae, *Saltator similis* is the most frequently described host species. *Isoospora* was unpublished in *Paroaria dominicana*, *Schistochlamys ruficapillus* and *Gnorimopsar chopi*. The new species of *Isoospora* described were *Isoospora dominicana*, *Isoospora ferri*, *Isoospora ruficapillus*, *Isoospora chopi* and *Isoospora gnorimopsar*. The new coccidian species and new passerine hosts add to the complexity of *Isoospora* in passerines and indicates for frequent monitoring during rehabilitation.

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