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Importance of Morphological and Morphometric Characteristics for the Diagnosis of Coccidia in the Saffron Finch, *Sicalis Flaveola*

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Abstract

The saffron finch, *Sicalis flaveola*, is a small, bright yellow Neotropical passerine native to South America, ranging from Argentina to Venezuela. The aim of the present study was to morphologically and morphometrically identify species of coccidia parasites of free-living and captive saffron finches in the states of Rio de Janeiro and Minas Gerais, Brazil. For this study, feces were collected from 30 captive birds and 30 free-living birds. Fecal samples were filtered through double gauze, mixed with 2.5% potassium dichromate ($K_2Cr_2O_7$), placed in a Petri dish and incubated at 23-28 °C until 70% of the oocysts were sporulated. Statistical analyses were performed using Microsoft Excel software. All birds analyzed were positive for coccidia, and oocysts of the species *Isospora sicalisi* and *Isospora cetasiensis* were found in the feces of captive birds. In the feces of free-living saffron finches, four different species of coccidia were identified, namely, *I. sicalisi*, *I. cetasiensis*, *Isospora bertoi* and *Eimeria flaveola*. It can be concluded that little is known about the diversity and distribution of coccidia in wild birds, requiring further study in different locations.

Keywords: Passeriformes, Feces, *Isospora* spp., *Eimeria* sp

1. Introduction

Saffron finches (*Sicalis flaveola*, Linnaeus, 1766) are found in all of South America, inhabiting open areas with sparsely distributed trees, including pastures, plantations and farms (Gwynne *et al*., 2010) [18]. The birds travel in pairs or small flocks, forage on the ground for insects and seeds, and visit feeders in suburban backyards (Rising, 2011) [33].

Coccidia are deleterious intestinal protozoan parasites that are widespread among domestic and wild birds. They can cause reduced weight gain, affect intestinal nutrient resorption, reduce fertility, or impact carotenoid-based plumage coloration (Long, 1982; Buchholz, 2004) [23, 8]. In birds, the most prevalent coccidian genera are *Isospora* Schneider, 1881 and *Eimeria*; however, *Isospora* spp. occur more frequently in passeriform birds, while *Eimeria* spp. are more frequent in nonpasseriform birds (Duszynski *et al*., 2000) [13].

The objective of the present study was to morphologically and morphometrically identify the species of parasitic coccidia of free-living and captive saffron finches, *S. flaveola*, in the municipalities of Campos dos Goytacazes, state of Rio de Janeiro, and Eugenopolis, state of Minas Gerais, Brazil.

2. Material and methods

A total of 60 saffron finches, *Sicalis flaveola*, were used, of which 30 were reared in cages at farms and establishments in the municipality of Campos dos Goytacazes, state of Rio de Janeiro, Brazil. The other 30 birds were free-living and inhabited peri-urban and rural regions of the municipality of Eugenopolis and were captured with mist nets. This study was approved by the Biodiversity Authorization and Information System (SISBIO) under protocol n° 78,016–1/2022, and all experimental protocols were approved by the ethics committee for the use of animals (protocol n° 523).

After capture, the birds were housed for 24 hours in cages with water and food provided *ad libitum*. The 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 Center for Advanced Research in Parasitology of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) in the municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil. Fecal samples from all birds were filtered through double gauze, mixed with 2.5% potassium dichromate ($K_2Cr_2O_7$), placed in a 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) [15].

Morphological observations and measurements, given in micrometers, were made using an Eclipse i80 optical microscope (Nikon, Japan). Photomicrographs were taken using a Coolpix 5700 digital camera (Nikon, Japan). Size ranges are given in parentheses, followed by the mean, standard deviation, and shape index (length/width).

The values observed for the largest diameter (LD), smallest diameter (SD) and morphometric index of the oocysts were graphically represented in histograms according to Sampaio (2002) [34], where the Y line represented the frequencies, while the X line represented the class ranges of oocyst measurements. The value of the class interval was obtained through the ratio of the amplitude of the values obtained in the sample by the ideal number of classes, which is represented by the product of multiplying the fourth root of the total number of samples by 2.5.

Simple linear regression analysis was applied to evaluate measurements of the smallest diameter (SD) over the largest diameter (LD) of sporulated oocysts belonging to a single species (Norton and Joyner, 1981) [28]. The SD measurements were organized on the Y line, and the LD measurements were organized on the X line. The graphs with the residuals, as well as the slope coefficients of the regression lines, were obtained using Microsoft Excel® software (Norton and Joyner, 1981; Sampaio, 2002) [28, 34].

3. Results and Discussion

All the birds analyzed were positive for coccidia, and in the feces of the captive birds, oocysts of the species *Isospora sicalisi* and *Isospora cetasiensis* were identified. The number of oocysts and the morphometric data measured for these species can be found in Table 1. In the feces of free-living saffron finches, four different species of coccidia were identified, namely, *I. sicalisi*, *I. cetasiensis*, *Isospora bertoi* and *Eimeria flaveola*. The morphometric data of the species of these genera can be found in Tables 2 and 3, respectively.

Table 1: Morphometry in micrometers of sporulated oocysts of *Isospora* species isolated from feces of captive saffron finches *Sicalis flaveola*

Measurements	Captivity			
	n[1]	*Isospora sicalisi*	n	*Isospora cetasiensis*
Oocyst				
Larger diameter	25	26.84±2.38 (23.21-32.68)	99	22.37±1.44 (19.04-27.72)
Smaller diameter	25	25.08±2.41 (21.34-31.38)	99	21.20±1.36 (18.7-24.88)
Morphometric index	25	1.07±0.06 (1.00-1.24)	99	1.06±0.05 (1.00-1.39)
Wall	25	1.19±0.20 (0.84-1.56)	54	1.04±0.11(0.80-1.44)
Sporocyst				
Larger diameter	42	17.54±1.40 (15.23-21.57)	136	15.63±1.13 (11.07-18.73)
Smaller diameter	42	12.14±1.20 (10.41-16.56)	136	10.80±0.80 (9.05-14.44)
Stieda body				
Width	34	2.15±0.53 (1.22-4.38)	57	2.04±0.37 (1.34-2.96)
Height	34	1.11±0.35 (0.45-2.68)	57	0.94±0.20 (0.60-1.54)
Substieda body				
Width	32	4.54±0.72 (2.37-5.88)	24	2.93±0.97 (2.10-6.23)
Height	32	2.73±0.46 (1.09-3.48)	24	1.77±0.67 (1.03-3.88)

[1]Number of measured oocysts.

Table 2: Morphometry in micrometers of sporulated oocysts of *Isospora* species isolated from feces of free-living saffron finches *Sicalis flaveola*

Measurements	Free Living									
		Isospora sicalisi				*Isospora cetasiensis*			n	*Isospora bertoi*
	n[1]	Without PG[2]	n	With PG	n	Without PG	n	With PG		
Oocyst										
Larger diameter	2	26.21±0.97 (25.24-27.18)	6	26.44±0.67 (25.31-27.40)	75	22.59±1.48 (18.66-26.58)	7	21.48±1.51 (19.23-23.45)	32	23.61±1.20 (21.14-26.54)
Smaller diameter	2	24.50±2.53 (21.97-27.03)	6	24.14±0.72 (22.79-25.22)	75	21.29±1.62 (17.80-25.33)	7	20.90±1.36 (18.66-22.70)	32	22.04±1.24 (19.41-24.56)
Morphometric index	2	1.08±0.07 (1.01-1.15)	6	1.10±0.05 (1.00-1.17)	75	1.06±0.05 (1.0-1.26)	7	1.03±0.02 (1.00-1.06)	32	1.07±0.05 (1.00-1.20)
Wall	-	-	1	0.96	17	1.19±0.13 (0.92-1.39)	3	1.29±0.07 (1.2-1.36)	18	1.12±0.10 (0.92-1.29)
Sporocyst										
Larger diameter	4	17.59±0.77 (16.32-18.29)	9	16.48±0.81 (15.23-17.76)	116	15.01±1.07 (10.42-17.92)	8	15.12±0.86 (13.85-16.52)	37	16.17±1.25 (13.61-17.86)
Smaller diameter	4	11.50±0.65 (10.92-12.53)	9	11.63±0.74 (10.55-13.16)	116	10.58±0.73 (9.08-13.68)	8	10.30±0.58 (9.60-11.32)	37	10.07±0.71 (8.94-12.43)
Stieda body										

Width	1	1.99	3	2.30±0.66 (1.61-3.19)	13	2.23±0.39 (1.49-2.9)	1	2.70	-	-
Height	1	0.93	3	0.93±0.22 (0.61-1.1)	13	0.88±0.11 (0.6-1.03)	1	1.17	-	-
Substieda body										
Width	1	3.46	2	4.17±0.87 (3.30-5.04)	6	2.83±0.28 (2.35-3.18)	1	3.00	-	-
Height	1	1.93	2	2.34±0.43 (1.91-2.76)	6	1.56±0.10 (1.39-1.68)	1	1.62	-	-
Polar granule										
Larger diameter	-	-	2	3.64±1.32 (2.32-4.95)	-	-	3	2.55±0.22 (2.18-2.74)	24	2.96±0.59 (1.90-4.06)
Smaller diameter	-	-	2	1.87±1.02 (0.85-2.89)	-	-	3	2.00±0.49 (1.36-2.73)	24	2.23±0.63 (1.12-3.64)

[1]Number of measured oocysts
[2]Polar granule

Table 3: Morphometry in micrometers of sporulated oocysts of *Eimeria flaveola* isolated from feces of free-living saffron finches *Sicalis flaveola*

Measurements	n[1]	*Eimeria flaveola*
Oocyst		
Larger diameter	150	22.67±1.57 (18.47-26.48)
Smaller diameter	150	21.19±1.42 (16.69-24.91)
Morphometric index	150	1.07±0.05 (1.00-1.25)
Polar granule		
Larger diameter	71	2.94±0.44 (1.83-3.96)
Smaller diameter	71	2.13±0.55 (1.20-3.68)
Sporocyst		
Larger diameter	104	14.24±1.71 (9.02-19.33)
Smaller diameter	104	9.98±1.02 (6.80-12.87)
Morphometric index	104	1.43±0.16 (0.74-2.11)
Sporocyst residuum		
Larger diameter	04	5.98±0.66 (5.12-6.82)
Smaller diameter	04	5.67±0.47 (5.15-6.20)
Stieda body		
Width	3	1.40±0.26 (1.19-1.77)
Height	3	0.59±0.12 (0.50-0.76)
Sporozoite		
Larger diameter	1	11.53
Smaller diameter	1	2.84
Nucleus		
Larger diameter	1	2.04
Smaller diameter	1	1.91
Refractile body		
Larger diameter	2	3.66±0.50 (3.16-4.15)
Smaller diameter	2	3.14±0.72 (2.42-3.85)

3.1 *Isospora sicalisi* Coelho *et al*., 2011

The sporulated oocysts of *I. sicalisi* found in the present study in both captive and free-living birds were characterized as subspherical to ellipsoid, smooth and with a double wall. Micropyle and oocyst residues were absent. Polar granules were absent in most oocysts (Figure 1a, b), but in some oocysts of free-living birds, ellipsoidal polar granules were observed (Figure 1c, d), a feature that has not yet been reported in the species. The sporocysts were ellipsoidal. The Stieda body had a button-like shape and trapezoidal substieda body. Sporocyst residue composed of scattered spherical granules of different sizes was observed. Vermiform sporozoites with a refractile body and nucleus were present.

Isospora sicalisi oocysts showed a higher frequency and tendency to have an LD between 25 to 26 µm (Figure 2a), an SD from 23 to 25 µm (Figure 2b) and a morphometric index between 1.0 and 1.1 (Figure 2c), showing a high tendency for oocysts of this species to be spherical to subspherical in shape. It was verified that in the simple linear regression, the points were close to the straight line with an R^2 value greater than 0.5, indicating that the oocysts morphologically similar to *I. sicalisi* also present morphometric similarities, confirming the diagnosis of the species (Figure 2d).

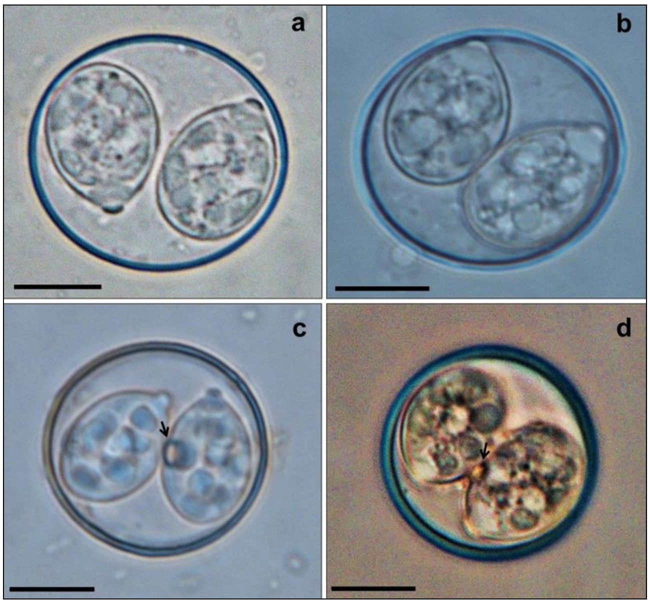

Fig 1: Optical micrograph of sporulated oocysts of *Isospora sicalisi* without polar granules (a and b) isolated from feces of the saffron finch, *Sicalis flaveola*, in captivity and free-living, and with polar granules (c and d) isolated from feces of free-living *S. flaveola*. Arrow indicates the polar granule. Bar: 10 µm.

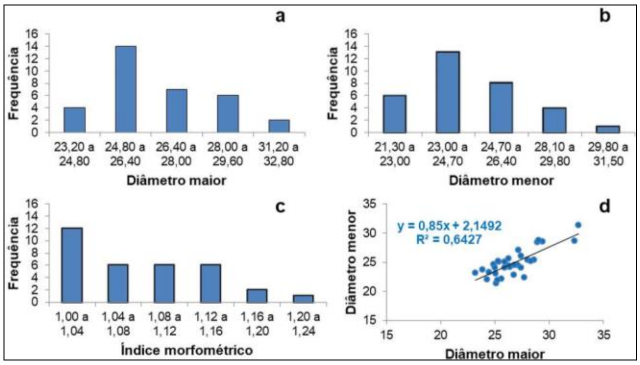

Fig 2: Histograms of the dimensions of the sporulated oocysts of *Isospora sicalisi* from *S. flaveola*: larger diameter (a), smaller diameter (b), morphometric index (c) and linear regression (d).

3.2 *Isospora cetasiensis* Coelho *et al*., 2011

The sporulated oocysts of *I. cetasiensis* found in captive and free-ranging birds were also subspherical to ellipsoidal, smooth and double walled. The micropyle and oocyst residue were absent. As in the species described above, the polar granule was absent in most oocysts (Figure 3a, b), but a subspherical to ellipsoidal polar granule was visualized in some oocysts isolated from free-living bird feces (Figure 3c, d). Oval-shaped sporocysts with a knob-shaped stieda body and a rounded substieda body were observed. Sporocyst residue composed of many scattered rounded granules of varying sizes was found. Vermiform sporozoites with a refractile body and nucleus were found.

Oocysts of *I. cetasiensis* have a greater tendency for an LD from 21 to 23 µm (Figure 4a) and an SD from 20 to 22 µm (Figure 4a). In the morphometric index histogram, it is observed that most oocysts have values from 1.0 to 1.1 (Figure 4c), which indicates their tendency to have a spherical to subspherical shape. *Isospora cetasiensis* oocysts have a certain proportionality between LD and SD dimensions, since linear regression resulted in an R^2 value above 0.5 (Figure 4d).

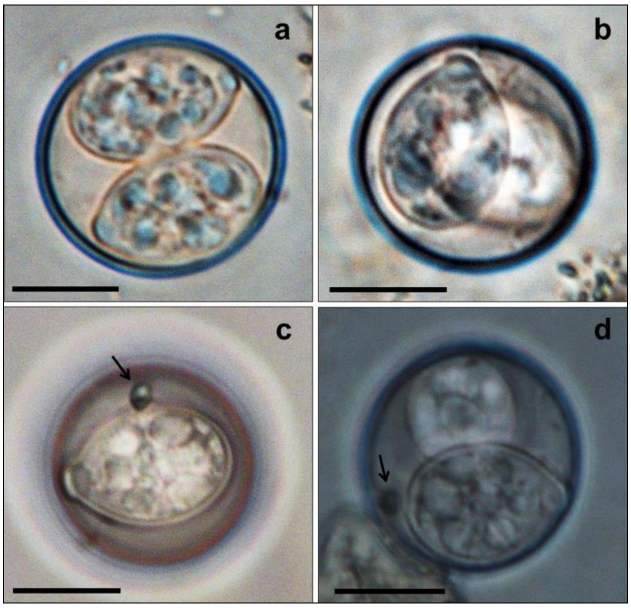

Fig 3: Optical micrograph of sporulated oocysts of *Isospora cetasiensis* without polar granules (a and b) isolated from feces of the saffron finch, *Sicalis flaveola*, in captivity and free-living, and with polar granules (c and d) isolated from feces of free-living *S. flaveola*. Arrow indicates the polar granule. Bar: 10 µm.

Through morphological and morphometric analyses, the present study confirmed the presence of the two species of Isospora described by Coelho *et al*. (2011) [11] in both captive and free-living saffron finches. However, in free-living birds, the presence of polar granules was observed in the oocysts of these two species, a characteristic not described by the authors. Thus, it was not possible to determine whether this characteristic is different from that of the species already described or a result of phenotypic variation. Therefore, this morphological structure is not very efficient for the identification of these species.

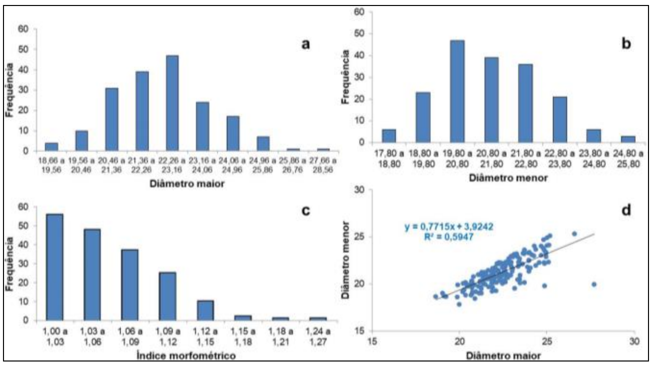

Fig 4: Histograms of the dimensions of sporulated oocysts of *Isospora cetasiensis* from *S. flaveola*: (a) larger diameter, (b) smaller diameter, (c) morphometric index and (d) linear regression.

3.3 *Isospora bertoi* Oliveira *et al*., 2023

Oocysts of the species *I. bertoi* (Fig 5) were found only in the feces of free-living birds. The sporulated oocysts were subspherical to ellipsoid with a double wall and a smooth outer surface. The micropyle and oocyst residue were absent; 1-2 polar granules of different shapes and sizes, which can be bilobed, were observed. The sporocysts had an elongated ellipsoid shape and were tapered at both ends. The stieda body was present in the form of a button, and the substieda body was absent. Compact sporocyst residue, composed of hundreds of granules scattered among the sporozoites, was observed. A claviform sporozoite with an elongated posterior refractile body and a nucleus was found.

According to the histograms of the oocyst measurements of *I. bertoi* (Fig 6), the frequencies in the classes increased and decreased, indicating fewer extreme values and more median values; therefore, the measured oocysts belonged to only one species.

For *I. bertoi*, it was observed that the oocysts had a greater tendency to have a 23.8 to 24.7 µm LD (Figure 6a) and 22 to 23 µm f SD (Figure 6b), since a higher frequency of oocysts was observed in these value classes. In the morphometric index histogram (Figure 6c), the frequencies are higher in the first three classes of lower values and decrease considerably in the classes of higher values. This result supports the high tendency for oocysts of this species to have a spherical to subspherical shape, that is, a morphometric index between 1.0 and 1.1. In the linear regression of the dimensions of the oocysts of *I. bertoi*, an R^2 value close to 0.5 was obtained (Figure 6d).

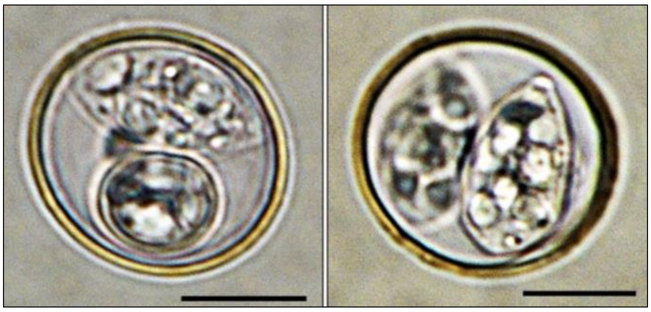

Fig 5: Optical micrograph of sporulated oocysts of *Isospora bertoi* isolated from feces of the free-living saffron finch, *Sicalis flaveola*. Bar: 10 µm.

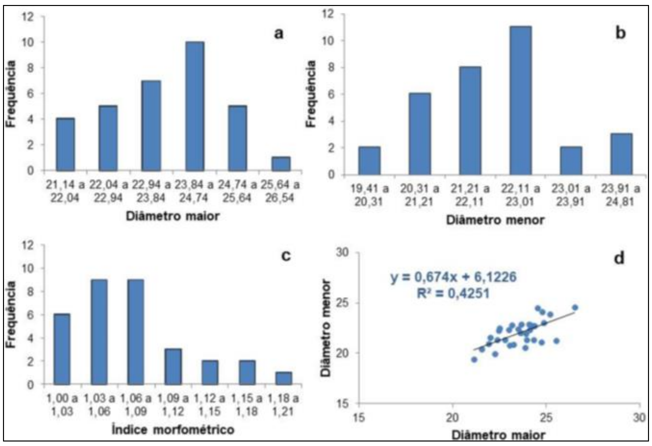

Fig 6: Histograms of the dimensions of sporulated oocysts of *Isospora bertoi* from *S. flaveola*: (a) larger diameter, (b) smaller diameter, (c) morphometric index and (d) linear regression.

The species *I. bertoi* presented morphology and morphometry distinct from those of the species described by Coelho *et al*. (2011) [11] and species found in New World passerine birds belonging to the Emberizidae family (Upton *et al*., 1985; McQuiston and Wilson, 1988; McQuistion and Wilson, 1989; McQuistion, 1990; Ball and Daszak, 1997; Carvalho-Filho, 2005; Silva *et al*., 2006; Berto *et al*., 2009; Balthazar *et al*., 2009; Pereira *et al*., 2011; Barreto, 2014) [8, 26, 27, 25, 1, 9, 37, 4, 2, 31, 3]. Differences in the morphological and morphometric characteristics of this coccidium were significant enough to assign it as a new species. In this way, a standard can be established for it.

3.4 *Eimeria flaveola* Gallo *et al*., 2022

This species was also observed only in the feces of wild birds. The sporulated oocysts (Fig 7) were subspherical with a double wall and a smooth external surface. The micropyle and oocyst residue were absent; a spherical to subspherical polar granule was present. The sporocysts were ovoid with a rounded end. The Stieda body was present in the form of a button and the substieda and parastieda bodies were absent. Sporocyst residue was present, forming a compact mass of granules. A claviform sporozoite with a refractile body and a rounded nucleus was observed.

In the histograms, the oocysts had a higher frequency between the values of 22.5 to 23.5 µm LD (Figure 8a) and 20.7 to 21.7 µm SD (Figure 8b), and in the histogram of the morphometric index, most oocysts had values from 1.0 to 1.1 (Figure 8c). The R^2 value was greater than 0.5; i.e., the data points were distributed close to the regression line (Figure 8d).

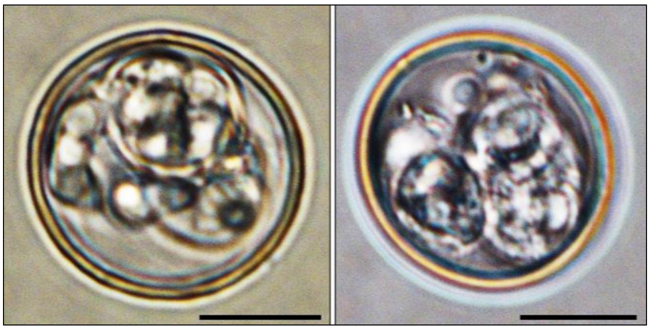

Fig 7: Optical micrograph of sporulated oocysts of *Eimeria flaveola* isolated from feces of the free-living saffron finch, *Sicalis flaveola*. Bar: 10 µm.

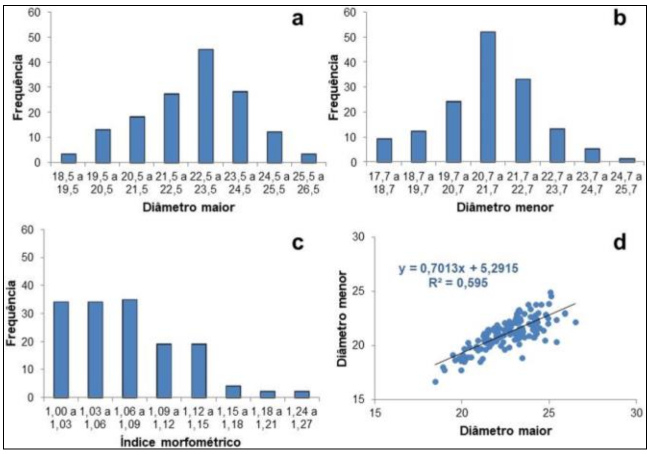

Fig 8: Histograms of dimensions of sporulated oocysts of *Eimeria flaveola*: (a) larger diameter, (b) smaller diameter, (c) morphometric index and (d) linear regression.

Descriptions of the genus *Eimeria* in the order Passeriformes are scarce. According to Duszynski and Wilber (1997) [15], a new coccidian species needs to be compared in detail with coccidian species that have similar characteristics and belong to the same host family. Twelve species of *Eimeria* spp. have been described with morphological and morphometric characteristics in the order Passeriformes (Labbé, 1896; Duszynski *et al*., 1999; Berto *et al*., 2008a; Berto *et al*., 2009; Soriano-Vargas *et al*., 2015, 2017; McAllister e Hnida, 2019) [22, 14, 6, 4, 35, 36, 24]. Of these, eight were found in Old World birds (Duszynski *et al*., 1999) [14], four were reported in New World birds (Duszynski *et al*., 1999) [14] and four reported in New World birds (Berto *et al*., 2008a; Berto *et al*., 2009; Soriano-Vargas *et al*., 2015, 2017; McAllister e Hnida, 2019) [6, 4, 35, 36, 24]. In addition, *Eimeria grallinida* was found in the bird *Grallina cyanoleuca* in Victoria, Australia, by Reece (1989) [32], who reported only stages of liver development. Sporulated oocysts had an average size of 15x10 µm, but morphological data were not provided.

Only one species of *Eimeria* has been described in the Emberizidae family, to which *S. flaveola* belongs (Soriano-Vargas *et al*., 2015, 2017) [35, 36]. *Eimeria atlapetesi* was described in *Atlapetes pileatus* Wagler in Mexico, and the oocysts (16.5 x 14.1 µm) and sporocysts (9.0 x 5.4 µm) were ellipsoid and smaller than those described in our research. It has a stieda body that varies from flat to half-moon in shape and a substieda body, which was not observed in the *E. flaveola* species found in the feces of free-living *S. flaveola*. Another difference is the absence of a nucleus in the sporozoite of the *E. atlapetesi* species.

The more structures that are analyzed in an oocyst, the more accurate the diagnosis becomes, hence the importance of associating morphometric and qualitative characters (Hassum *et al*., 2007) [19]. Norton and Joyner (1981) [28] distinguished *E. acervulina* from *E. mivati*, parasites of birds, through regression analysis of data referring to the LD and SD of oocysts. Pereira *et al*. (2001) [30] compared the angular coefficients of linear regression between the LD and SD of *Hammondia heydorni* oocysts to characterize the intraspecific variations in different hosts. Linear regression analysis was employed in the present study to assess possible inter- and intraspecific morphometric differences in the LD and SD of oocysts.

In the linear regression graphs of the species *I. cetasiensis*, *I. sicalisi* and *E. flaveola* generated in the present study, the points remained close to each other and to the regression line. Thus, there were few variations in SD on LD, corroborating the findings of Berto *et al*. (2011) [7]. On the other hand, the R^2 value obtained after constructing the linear regression for *I. bertoi* oocysts was less than 0.5. Thus, several variations can occur in SD and LD, so a pattern cannot be established for *I. bertoi* oocysts. According to Berto *et al*. (2011) [7], these variations may occur due to a possible polymorphism of the species. Several factors could be associated with this characteristic, such as host stress, immunity and nutrition; the infecting dose (Fayer, 1980; Joyner, 1982; Berto *et al*., 2008b) [16, 20, 5]; the stage of the patent period when oocysts have been eliminated, since the oocysts of some coccidia can vary in size by more than 40% during patency (Duszynski, 1971, Joyner and Long, 1974; Catchpole *et al*., 1975) [12, 21, 10]; and phenotypic plasticity, that is, when coccidia activate different phenotypes in response to their environment

(Parker and Duszynski, 1986, Gardner and Duszynski, 1990; Berto *et al*., 2008b) [29, 17, 5]. A second justification would be supported by the ellipsoid shape of the oocyst, which is a three-dimensional structure measured under a light microscope in a dimensional way. Thus, an ellipsoid oocyst, depending on its position under the coverslip, that is, the angle of observation, can appear spherical to ovoid/ellipsoid, while a spherical oocyst, regardless of the angle of observation, will always have the same dimensions.

4. Conclusion

The identification in the present study of species of coccidia recently reported in the scientific literature and observed here, in addition to morphological characteristics not yet described, emphasizes how little is known about the diversity and distribution of coccidia in wild birds. In addition, the identification of coccidia species reported in a different and distant location from that in the present study demonstrates the wide distribution and dispersion of wild bird coccidia in southeastern Brazil. Thus, the municipalities of Eugenopolis, Minas Gerais and Campos dos Goytacazes in the state of Rio de Janeiro can be considered new locations for coccidian parasitism in the saffron finch *S. flaveola*.

5. References

1. Ball SJ, Daszak P. *Isospora tiaris* n. sp. (Apicomplexa: Eimeriidae) from the sooty grassquit (*Tiaris fuliginosa*), a passeriform bird of South America. Journal of Parasitology. 1997; 83(3):465-466.
2. Balthazar LMC, Berto BP, Flausino W, Lopes CWG. *Isospora ticoticoi* n. sp. (Apicomplexa: Eimeriidae) from the rufous-collared sparrow *Zonotrichia capensis* in South America. Acta Protozoologica. 2009; 48(4):345-349.
3. Barreto C. Ocorrência e identificação de coccídeos em amostras fecais de passeriformes silvestres (Aves: Passeriformes) no Centro de Triagem de Animais Silvestres do IBAMA em Belo Horizonte, 2014, p57.
4. Berto BP, Balthazar LMC, Flausino W. Lopes CWG. Three new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the buffy-fronted seedeater *Sporophila frontalis* Verreaux, 1869 (Passeriformes: Emberizidae) from South America. Systematic Parasitology. 2009; 73(4):65-69.
5. Berto BP, Flausino W, Almeida CRR, Lopes CWG. Polymorphism of *Tyzzeria parvula* (Kotlán, 1933) Klimes, 1963 (Apicomplexa: Eimeriidae) oocysts from the greylag geese *Anser anser* L., 1758 conditioned in two distinct sites. Revista Brasileira de Medicina Veterinária. 2008b; 30(4):215-219.
6. Berto BP, Flausino W, Ferreira I, Lopes CWG. *Eimeria divinolimai* sp. n. (Apicomplexa: Eimeriidae) in the rufous casiornis *Casiornis rufus* Vieillot, 1816 (Passeriformes: Tyrannidae) in Brazil. Revista Brasileira de Parasitologia Veterinária. 2008a; 17:33-35.
7. Berto BP, Flausino W, Mcintosh D, Teixeira-Filho WL, Lopes CWG. Coccidia of New World passerine birds (Aves: Passeriformes): A review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). Systematic Parasitology. 2011; 80:159-204.
8. Buchholz R. Effects of parasitic infection on mate sampling by female Wild Turkeys (*Meleagris gallopavo*): Should infected females be more or less choosy? Behavioral Ecology. 2004; 15:687-694.
9. Carvalho-Filho P, Meireles G, Ribeiro C, Lopes CWG. Three new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the double-collared seedeater, *Sporophila caerulescens* (Passeriformes: Emberizidae), from eastern Brazil. Memórias do Instituto Oswaldo Cruz. 2005; 100(2):151-154.
10. Catchpole J, Norton CC, Joyner LP. The occurrence of *Eimeria weybridgensis* and other species of coccidia in lambs in England and Wales. British Veterinary Journal. 1975; 131(4):392-401.
11. Coelho CD, Berto BP, Neves DM, Oliveira VM, Flausino W, Lopes CWG. Two new *Isospora* species from the saffron finch, *Sicalis flaveola* in Brazil. Acta Parasitologica. 2011; 56:239-244.
12. Duszynski DW. Increase in size of *Eimeria separata* oocysts during patency. Journal of Parasitology. 1971; 57(5):948-952.
13. Duszynski DW, Couch L, Upton SJ. The Coccidia of the World, 2000. Retrieved from: https://www.k-state.edu/parasitology/worldcoccidia. Accessed Mar 1, 2022
14. Duszynski DW, Upton SJ, Couch L. The coccidia of Passeriformes (*Eimeria*), 1999. Retrieved from: http://eimeria.unl.edu/passer.html. Acessed September 15, 2021
15. Duszynski D, Wilber PG. A guideline for the preparation of species descriptions in the Eimeriidae. Journal of Parasitology. 1997; 83(2):333-336.
16. Fayer R. Epidemiology of protozooan infection: The Coccidia. Veterinary Parasitology. 1980; 6(1-3):75-103.
17. Gardner SL, Duszynski DW. Polymorphism of eimerian oocysts can be a problem in naturally infected hosts: An example from subterranean rodents in Bolivia. Journal of Parasitology. 1990; 76(6):805-811.
18. Gwynne JA, Ridgely RS, Tudor G, Argel M. Aves do Brasil. vol. 1: Pantanal and Cerrado. São Paulo, Editora Horizonte, 2010.
19. Hassum IC, Valladares GS, Menezes RCAA. Diferenciação das espécies de *Eimeria* parasitas de ovinos pelo uso da regressão linear e algoritmos morfológicos. Revista Brasileira de Parasitologia Veterinária. 2007; 16(2):97-104.
20. Joyner LP. Host and Site specificity. In. Long PL. The biology of the Coccidia. Baltimore, University Park Press, 1982, 35-62.
21. Joyner LP, Long PL. The specific characters of the *Eimeria*, with special reference to the coccidia of the fowl. Avian Pathology, 1974; 3(3):145-57.
22. Labbé A. Recherches zoologiques, cytologiques et biologiques sur less coccidies. Archives de Zoologie Experimentale et Generale.1896; 24:517-654.
23. Long PL. The biology of the Coccidia. London, UK, Edward Arnold Publishers Ltd, 1982.
24. McAllister CT, Hnida JAA. New *Eimeria* (Apicomplexa: Eimeriidae) from the Barn Swallow, *Hirundo rustica* (Aves: Passeriformes: Hirundinidae), in Southeastern Oklahoma: The Fourth *Eimeria* Species from New World Passeriformes. Journal of Parasitology. 2019; 105(5):693-696.

25. McQuistion TE. *Isospora daphnensis* n. sp. (Apicomplexa: Eimeriidae) from the medium ground finch (*Geospiza fortis*) from the Galapagos Island. Journal of Parasitology. 1990; 76(1):30-32.
26. McQuistion TE, Wilson M. Four new species of *Isospora* from the small tree finch (*Camarhynchus parvulus*) from the Galapagos Island. Journal of Parasitology. 1988; 35(1):98-99.
27. McQuistion TE, Wilson M. *Isospora geospizae*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the small ground finch (*Geospiza fuliginosa*) and the medium ground finch (*Geospiza fortis*) from the Galapagos Island. Systematic Parasitology. 1989; 14(2):141-44.
28. Norton CC, Joyner LP. *Eimeria acervulina* and *E.mivati*: oocysts, life-cycle and ability to develop in thechicken embryo. Parasitology. 1981; 83(2):269-279.
29. Parker BB, Duszynski DW. Polymorphism of eimerian oocysts: A dilemma posed by working with some naturally infected hosts. Journal of Parasitology. 1986; 72(4):602-604.
30. Pereira MJS, Fonseca AH, Lopes CWG. Regressão linear na caracterização de variações morfométrica sem coccidia. Revista Brasileira de Parasitologia Veterinária. 2001; 10(2):75-78.
31. Pereira LQ, Berto BP, Flausino W, Lovato M, Lopes CWG. *Isospora bocamontensis* n. sp. (Apicomplexa: Eimeriidae) from the yellow cardinal *Gubernatrix cristata* (Vieillot) (Passeriformes: Emberizidae) in South America. Systematic Parasitology. 2011; 78(1):73-80.
32. Reece RL. Hepatic coccidiosis (*Eimeria sp*) in a wild magpie-lark (*Grallina cyanoleuca*), Avian Pathology. 1989; 18(2):357-362.
33. Rising JD. Family Emberizidae (buntings and new world sparrows). In: Del Hoyo J *et al*., ed. Handbook of the Birds of the World. Vol. 16, Tanagers to New World Blackbirds. Barcelona, Spain, Lynx Edicions, 2011, 633-634.
34. Sampaio IBM. Estatística aplicada à experimentação animal. Belo Horizonte, Fundação de Ensino e Pesquisa em Medicina e Zootecnia (FEPMVZ), 2002.
35. Soriano-Vargas E, Medina JP, Salgado-Miranda C, Garcia-Conejo N, Galindo-Sanchez KP, Janczur MK, *et al*. *Eimeria pileata* n. sp. (Apicomplexa: Eimeriidae) from the rufous-capped brush finch *Atlapetes pileatus* Wagler (Passeriformes: Emberizidae) in Mexico. Systematic Parasitology. 2015; 92:261-265.
36. Soriano-Vargas E, Salgado-Miranda C, Zepeda-Velázquez AP, Medina JP, Janczur MK, González-Gómez M, *et al*. *Eimeria atlapetesi* nom. nov., a replacement name for *Eimeria pileata* Soriano-Vargas *et al*., 2015 (Apicomplexa: Eimeriidae), preoccupied by *Eimeria pileata* Straneva and Kelley, 1979 (Apicomplexa: Eimeriidae), with observations on histopathology and phylogenetic analysis. Zootaxa. 2017; 4227(1):144-150.
37. Silva EAT, Literák I, Koudela B. Three new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the lesser seed-finch, *Oryzoborus angolensis* (Passeriformes: Emberizidae) from Brazil. Memórias do Instituto Oswaldo Cruz. 2006; 101(5):573-576.
38. Upton SJ, Current WL, Clubb SL. Two new species of *Isospora* (Apicomplexa: Eimeriidae) from passeriform birds of South America. Systematic Parasitology. 1985; 7(3):227-229.