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Report of coccidiosis in a free-living green-winged saltator *Saltator similis* in Itatiaia National Park in southeastern Brazil[1]

Carla Maronezi[2], Carlos N. Ortúzar-Ferreira[3], Lucas A.S. Andrade[3], Carolina M.S. Caruncho[4], Mariana S. Oliveira[3] and Bruno P. Berto*[5]

ABSTRACT.- Maronezi C., Ortúzar-Ferreira C.N., Andrade L.A.S., Caruncho C.M.S., Oliveira M.S. & Berto B.P. 2024. **Report of coccidiosis in a free-living green-winged saltator *Saltator similis* in Itatiaia National Park in southeastern Brazil**. *Pesquisa Veterinária Brasileira 44:e07451, 2024*. Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23897-000, Brazil E-mail: bertobp@ufrrj.br

The green-winged saltator *Saltator similis* (d'Orbigny & Lafresnaye, 1837) is a passerine bird highly valued by bird breeders and a target of illegal wildlife trafficking. Coccidian chromists are etiological agents of coccidiosis, which may be asymptomatic and enzootic in bird populations when environmental conditions are favorable. However, epizootic outbreaks with severe disease can occur due to environmental changes. In this context, this study reports a case of coccidiosis in a free-living juvenile specimen of *S. similis* captured at the Itatiaia National Park in southeastern Brazil. Typical clinical signs of coccidiosis were observed, and it was associated with greenish mucoid diarrhea containing a high density of coccidian oocysts. Three *Isospora* spp. were specifically identified in 14 fecal samples (fecal droplets) collected over two hours. *Isospora saltatori* (Berto, Balthazar, Flausino & Lopes, 2008) had the highest density and positivity throughout most of the collection period. Finally, this paper discusses the importance of the continuous evaluation of the health of birds as bioindicators and their coccidian ecological biomarkers as one of the strategies for evaluating the conservation status of Itatiaia National Park.

INDEX TERMS: Coccidia, green-winged saltator, *Saltator similis,* conservation, isosporosis, oocysts, Parque Nacional do Itatiaia.

RESUMO.- [Relato de coccidiose em um trinca-ferro *Saltator similis* de vida-livre no Parque Nacional do Itatiaia no sudeste brasileiro.] O trinca-ferro *Saltator similis* (d'Orbigny & Lafresnaye, 1837) é um pássaro muito valorizado por criadores de aves e pelo tráfico ilegal de animais silvestres. Os cromistas coccídios são agentes etiológicos da coccidiose, a qual pode ser assintomática e enzoótica em populações de aves quando as condições ambientais são favoráveis, mas surtos epizoóticos com doença grave podem ocorrer como resultado de mudanças ambientais. Nesse contexto, este artigo relata um caso de coccidiose em um espécime juvenil de vida livre de *S. similis* capturado no Parque Nacional de Itatiaia, no sudeste do Brasil. Foram observados sinais clínicos típicos de coccidiose, associados a diarreia mucóide esverdeada contendo alta densidade de oocistos. Três *Isospora* spp. foram especificamente identificadas em 14 defecações coletadas durante um período de duas horas. *Isospora saltatori* (Berto, Balthazar, Flausino & Lopes, 2008) foi a espécie com maior densidade e positividade durante a maior parte do período de coleta. Por fim, este artigo discute a importância da avaliação contínua da saúde das aves como bioindicadores, e de seus biomarcadores ecológicos coccídios, como uma

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[2] Graduate Program in Science, Technology and Innovation in Agriculture, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, RJ 23897-000, Brazil.

[3] Graduate Program in Animal Biology, Instituto de Ciências Biológicas e da Saúde (ICBS), Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, RJ 23897-000, Brazil.

[4] Veterinary Medicine Course, Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, RJ 23897-000, Brazil.

[5] Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde (ICBS), Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, RJ 23897-000, Brazil. *Corresponding author: bertobp@ufrrj.br

das estratégias para avaliação do estado de conservação do Parque Nacional de Itatiaia.

TERMOS DE INDEXAÇÃO: Coccidia, trinca-ferro, *Saltator similis,* conservação, isosporose, oocistos, Parque Nacional do Itatiaia.

INTRODUCTION

Coccidiosis is among the main parasitic diseases affecting domestic and wild animals. It is a cosmopolitan disease, prevalent in several ecosystems or animal production systems, and is reported in a wide range of hosts of all groups of vertebrates (Duszynski 2021).

Coccidians, the etiologic agents of coccidiosis, are obligate intracellular parasitic chromists that can cause intense intestinal (and possibly extraintestinal) parasitism in their hosts (Box et al. 1981, Ruggiero et al. 2015). The main clinical sign of coccidiosis is diarrhea, which may be secondarily accompanied by dehydration, prostration and cachexia (Atkinson et al. 2008). In galliform birds, the genus *Eimeria* (Schneider, 1875) is widely studied in chickens, turkeys, guinea fowl, etc. because some *Eimeria* spp. cause severe intestinal disease that consequently impairs weight gain; therefore, being of great importance for poultry farming (Swayne et al. 2020). In passerine birds, this disease is mainly caused by coccidians of the genus *Isospora* (Schneider, 1881), which is why it is also known as isosporosis (Atkinson et al. 2008). The diversity and distribution of *Isospora* spp. from Passeriformes is abundant, having been continuously described and reported since the 19th century from all continents of the world and many families and species (Berto et al. 2011).

The green-winged saltator *Saltator similis* (d'Orbigny & Lafresnaye, 1837) is a passerine of the order Passeriformes, family Thraupidae and subfamily Saltatorinae, which has a wide geographic distribution in the tropical and subtropical ranges of South America (BirdLife International 2018). This species has a melodious song and is therefore highly valued by bird breeders and the target of illegal wildlife trafficking (Lopes et al. 2013, Maronezi et al. 2022). Three coccidian species are recorded from *S. similis*: *Isospora saltatori* (Berto, Balthazar, Flausino & Lopes, 2008), *Isospora trincaferri* (Berto, Balthazar, Flausino & Lopes, 2008), and *Isospora similisi* (Coelho, Berto, Neves, Oliveira, Flausino & Lopes, 2013). These *Isospora* spp. were recently characterized morphologically and molecularly from green-winged saltators from captivity near the "Parque Nacional de Itatiaia" (Itatiaia National Park) in southeastern Brazil (Maronezi et al. 2022). In this context, the aim of the current study was to report a case of coccidiosis in a free-living specimen of *S. similis* captured in "Parque Nacional de Itatiaia", a federal conservation unit of high ecological relevance in southeastern Brazil.

MATERIALS AND METHODS

Animal Ethics. Field-collecting permits were issued by the "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBio - Chico Mendes Institute for Biodiversity Conservation) through the "Sistema de Autorização e Informação em Biodiversidade" (SISBIO - Biodiversity Authorization and Information System) under license number 70132, and Animal Ethics Committee (CEUA) of the "Universidade do Grande Rio" (UNIGRANRIO) under protocol number 021/2019. Banding permits and metal rings were issued by the "Centro Nacional de Pesquisa e Conservação de Aves Silvestres"(CEMAVE - Brazil's National Center for Wild Bird Research and Conservation, Senior Ringer: BPB, registration 5967850).

Study site, capture and evaluation of wild birds and sample collection. From July 1 to 3, 2022, an expedition was carried out in "Parque Nacional de Itatiaia" (Itatiaia National Park), specifically along the 5th km of the "Travessia Ruy Braga" (Ruy Braga Crossing) (22°25'39.9'' S; 44°37 '49.4'' W; altitude 1,378m), which is a 21km trail that leads from the lower part to the high plateau of the park, with an elevation gain of about 1,400m. The purpose of this expedition was to capture wild birds using mist nets, evaluate them, band them and collect fecal samples. On the first two days, the mist nets were open from 9 a.m. to 6 p.m., and on the third day, only in the morning, from 9 a.m. to 12 p.m., totaling approximately 21 hours of bird capture on this expedition. Among birds of different orders, families and species captured, only one green-winged saltator, *Saltator similis* was captured, a species infrequently captured even in preserved environments such as the "Parque Nacional de Itatiaia". This *S. similis* specimen was banded on the leg with a metal ring numbered 'F61663' provided by CEMAVE, photographed, measured, evaluated, and its biometrical (total length and mass), biological (sex, life stage, clinical signs, molt, accumulated fat content and incubation plate) and ecological (presence of ectoparasites such as ticks, lice, mites, etc.) data were recorded, following the guidelines proposed by Sousa & Serafini (2020). The bird was then kept in a box lined with clean paper for two hours to collect its droppings. During this period, the paper was examined and replaced several times to obtain fresh droplets of feces separately (Fig.1-4). Each fresh droplet of feces was placed individually in a centrifuge tube with a solution of 2.5% potassium dichromate ($K_2Cr_2O_7$) (Dolnik 2006). After these collections, the bird was released at the same site of its capture.

Recovery and quantification of coccidian oocysts from fecal droplets and morphological study. Samples were examined at the "Laboratório de Biologia de Coccídios" (LABICOC - Laboratory of Coccidia Biology) of the "Universidade Federal Rural do Rio de Janeiro"(UFRRJ). All the samples were incubated at room temperature (25°C) for seven days. Oocysts were isolated by flotation in Sheather's sugar solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski & Wilber (1997) and Berto et al. (2014). The oocysts were quantified as estimates of the number of oocysts per fecal drop (OPD), by counting the total number of oocysts recovered from a dropping on a microscope slide, following the guidelines of Dolnik (2006). This OPD number coincided with the parasite density (Bush et al. 1997). Morphological observations were made, and photomicrographs were taken using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with an Eurekam 5.0 digital camera (BEL Photonics, Monza, Italy). Figures were edited using two software programs (Corel DRAW and Corel PHOTO-PAINT) from CorelDRAW® (Corel Draw Graphics Suite, Version 2020, Corel Corporation, Canada).

RESULTS

The green-winged saltator *Saltator similis* captured in this study measured 221mm in total length, 48.6g in mass, without incubation plate, had no molt, moderate content of accumulated fat, and was free of ectoparasites. Typical clinical signs of coccidiosis were observed during this evaluation process, such as ruffled feathers and cloaca and tail feathers smeared with diarrheal feces (Fig.1-4). Additionally, the specimen was identified as of indeterminate sex and juvenile

life stage based on the observation of the presence of labial commissure, beak depigmentation and fairly dull plumage with little pigmentation (Fig.1 and 2) (Sousa & Serafini 2020).

Fourteen fecal samples (fecal droplets) were obtained from this juvenile specimen of *S. similis* in two hours between 4 and 6 p.m. on the first day of July 2022. All these fecal droplets were of greenish mucoid diarrhea. Of the 14 fecal droplets, 13 (93%) tested positive for oocysts of *Isospora* spp. (Table 1). Parasite density per fecal droplet ranged from 3 to 6,500 oocysts, with a mean of 1,454. Eight fecal droplets contained more than 100 oocysts, and 20,361 oocysts were shed in these two hours of collection (14 fecal droplets) (Table 1).

In this study, three *Isospora* spp. were specifically identified in the fecal droplets collected from the juvenile specimen of *S. similis*. This identification was based on a morphological comparison with the recent work by Maronezi et al. (2022): *Isospora saltatori*, *I. trincaferri* and *I. similisi* (Fig.5-10). *Isospora saltatori* was the species with the highest density and positivity, followed by *I. trincaferri* and *I. similisi* (Table 1). Figure 11 shows the variation of these three *Isospora* spp. over the two hours of sequential fecal droplet collection. As can be seen, *I. saltatori* was denser most of the time, albeit superseded by *I. trincaferri* at a few moments (in 4 of 14 fecal droplets) at the beginning and end of the collection period. Overall, there were two peaks of oocyst shedding, one in the first hour (2nd and 3rd fecal droplets) and another in the second hour (11th fecal droplet), which comprised 83% of the oocysts that were shed.

Photographs of this juvenile specimen of *S. similis* of this study, symptomatic for coccidiosis, its diarrheal fecal droplets, as well as photomicrographs of oocysts from the three *Isospora*

Fig.1-4. (**1**) Specimen of *Saltator similis* captured in the 5th km of the "Travessia Ruy Braga" of the "Parque Nacional de Itatiaia", identified as juvenile due to the presence of labial commissure (arrowheads), beak depigmentation (arrows) and fairly dull plumage with little pigmentation. The specimen showed typical clinical signs of coccidiosis, such as (**1**) ruffled feathers and (**2**) cloaca and tail feathers smeared with diarrheal feces. (**3**) Samples were collected by fecal droplets shed on clean paper, (**4**) continuously examined and replaced for two hours when the specimen was kept in an appropriate box.

Table 1. Positivity and specific density of *Isospora* spp. in fecal samples (fecal droplets) collected (between 4 and 6 p.m.) from a free-living green-winged saltator *Saltator similis* with clinical signs of coccidiosis in Itatiaia National Park in southeastern Brazil

Sample (fecal droplet)	Oocysts per droplet – OoPD (total and specific density)			
	Isospora saltatori	*Isospora trincaferri*	*Isospora similisi*	Total
1st	2	0	1	3
2nd	2,708	1,250	1,042	5,000
3rd	2,826	2,543	1,130	6,500
4th	13	4	13	31
5th	438	984	328	1,750
6th	16	16	20	52
7th	82	14	41	137
8th	0	0	0	0
9th	8	5	10	23
10th	41	51	10	102
11th	2,250	2,000	1,250	5,500
12th	174	348	58	580
13th	274	187	159	620
14th	11	29	23	63
Positivity	13/14 (93%)	12/14 (86%)	13/14 (93%)	13/14 (93%)
Mean density	632	531	292	1,454
Total density	8,843	7,431	4,085	20,361

spp. identified, are available in the Parasitology Collection of LABICOC at UFRRJ, under Repository No. 134/2023[6].

DISCUSSION

Coccidiosis is rarely found in free-living birds, unlike what is observed in captive birds, which commonly suffer from this parasitosis (Coelho et al. 2013, Vasconcellos et al. 2013, Oliveira et al. 2018, Barreto et al. 2020). Although it is difficult to understand precisely disease dynamics and the impacts of pathogens in wild animals (Lachish & Murray 2018), this observation can be explained by the fact that, in a conserved natural environment that is in ecological equilibrium, coccidians are part of the intestinal biota of their immunocompetent hosts, without causing major damage, since infected birds are almost always asymptomatic (Berto & Lopes 2020). On the other hand, birds in altered wild environments or kept in confinement or captivity (by legal breeders or by illegal wildlife trafficking) are highly susceptible to coccidians and other opportunistic etiological agents due to the low immunity resulting from chronic stress imposed by unfavorable environmental conditions (Coelho et al. 2013, Vasconcellos et al. 2013, Campos et al. 2017, Berto & Lopes 2020).

It is also worth mentioning that in cases of severe coccidiosis, wild animals suffering from clinical signs tend to move less and, therefore, are difficult to capture. This condition can lead to imprecision in disease ecology studies (Lachish & Murray 2018). In this sense, continuous monitoring of coccidiosis in conservation areas, which can detect an increase in the density of coccidian oocysts early, can provide support for decision-making aimed at environmental conservation before the animals can suffer severely as a result of any environmental disturbance (Berto & Lopes 2020). In this context, despite the "Parque Nacional de Itatiaia" being an integral protection unit in an excellent state of conservation, any observation that may indicate early environmental changes should be evaluated; as in this case, clinical signs in a bioindicator bird and high densities of its ecological biomarker coccidians (Berto & Lopes 2020). At the same time, it should also be noted that the green-winged saltator captured here was a juvenile specimen. Nesting and juvenile passerines are more susceptible to diseases in general since their immune system is still under development; therefore, the clinical signs of coccidiosis easily observed in our specimen are presumably related to its life stage (Dolnik 1998, Schoener et al. 2013, Berto & Lopes 2020). Nevertheless, this report is highly relevant in the context of the "Parque Nacional de Itatiaia" conservation, given that clinical signs associated with coccidiosis in wild birds living in conserved forests are rarely found even among juvenile passerines (Dolnik et al. 2010).

The finding of the clinical signs attributed to coccidiosis was reinforced by the high density of *Isospora* spp. oocysts in the bird's fecal droplets. This large number of more than 20,000 oocysts would be shed into the environment that this specimen of *Saltator similis* moved around in, obviously considering that the symptoms of coccidiosis did not affect the bird's fitness, and hence, its mobility in this period from 4 p.m. to 6 p.m., when the samples were collected. In this context, it is worth mentioning that the time window during which the bird's droppings were collected in this study was the most advisable since the collection of fecal samples in the late afternoon is a priority in studies about the prevalence and density of *Isospora* spp. of passerine birds, given the recognized diurnal periodicity of oocyst shedding, which is exponentially higher in the late afternoon (Dolnik et al. 2010, Coelho et al. 2013, Biard et al. 2022). It is known that the success of parasitism of *Isospora* spp., which is fecal-oral, depends on the number of their exogenous infectious forms (oocysts) shed into the environment and their efficiency in being ingested by the next susceptible host. Therefore, this specimen of *S. similis* is a potential transmitter/disperser

[6] Available at <http://r1.ufrrj.br/labicoc/colecao.html> Accessed on Apr. 5, 2024.

of *Isospora* spp. due to the large number of oocysts shed at a peak feeding time (and possibly location) of susceptible *Saltator* spp. (Fayer 1980, Dolnik et al. 2010, Coelho et al. 2013, Berto & Lopes 2020, Biard et al. 2022). Moreover, since the infecting dose is presumably directly associated with the severity of coccidiosis, the shedding of large numbers of oocysts and consequent ingestion by susceptible hosts can cause epizootics and increase morbidity and mortality in wild birds (Fayer 1980, Berto & Lopes 2020).

The positivity and specific densities of the three *Isospora* spp. identified in this study (Table 1) differed from the findings reported by Coelho et al. (2013). Those authors observed a higher density and prevalence of *I. similisi*, followed by *I. saltatori* and *I. trincaferri*, in green-winged saltators rescued from illegal wildlife trafficking kept in quarantine in a wildlife rehabilitation center in the municipality of Seropédica (Centro de Triagem de Animais Silvestres - CETAS/Seropédica) in the state of Rio de Janeiro, Brazil. In these two studies, the prevalence (positivity) and density of *I. saltatori* were consistent. In comparison, those of *I. similisi* and *I. trincaferri* were complete opposites in these two studies, the only ones reporting specific prevalence rates and densities of *Isospora* spp. shed by *Saltator* spp. In this regard, it is possible that the dissimilarity of the environmental conditions in these studies (free-living and rescued from illegal wildlife trafficking) to which these birds were subjected may have influenced the prevalence and specific densities of these three *Isospora* spp. from *Saltator* spp. (Berto & Lopes 2020).

Despite the currently valid taxonomic record of the three *Isospora* spp. from *S. similis* (Maronezi et al. 2022), little is known about the pathogenicity of these species, prepatent/patent periods, or even their lifecycle patterns. Oliveira et al. (2018) reported extraintestinal isosporosis in *S. similis*, assessing the pathological and molecular characteristics of the disease. Pathological findings included hepatomegaly, splenomegaly, necrosis of lymphoid follicles, hepatic necrosis, and severe enteritis, with merozoites found in the heart, small intestine, proventriculus, brain, liver, spleen and kidneys. Molecular identification of merozoites in this study (Oliveira et al. 2018) indicated a similarity of 99% with an *Isospora* sp. by the 23s gene and of 97% with *Isospora greineri* (Hafeez, Stasiak, Delnatte, El-Sherry, Smith & Barta, 2014) by the mitochondrial *cox1* gene. However, despite the significant contribution of that study, no oocyst was morphologically or specifically identified. Campos et al. (2017) also reported extraintestinal coccidiosis in *S. similis* but, like this study, were limited in their diagnosis of atoxoplasmosis (systemic/extraintestinal isosporosis) by the finding of merozoites in peripheral blood and hematological changes (leukocytosis, monocytosis, and lymphocytosis), without specifically identifying the etiological

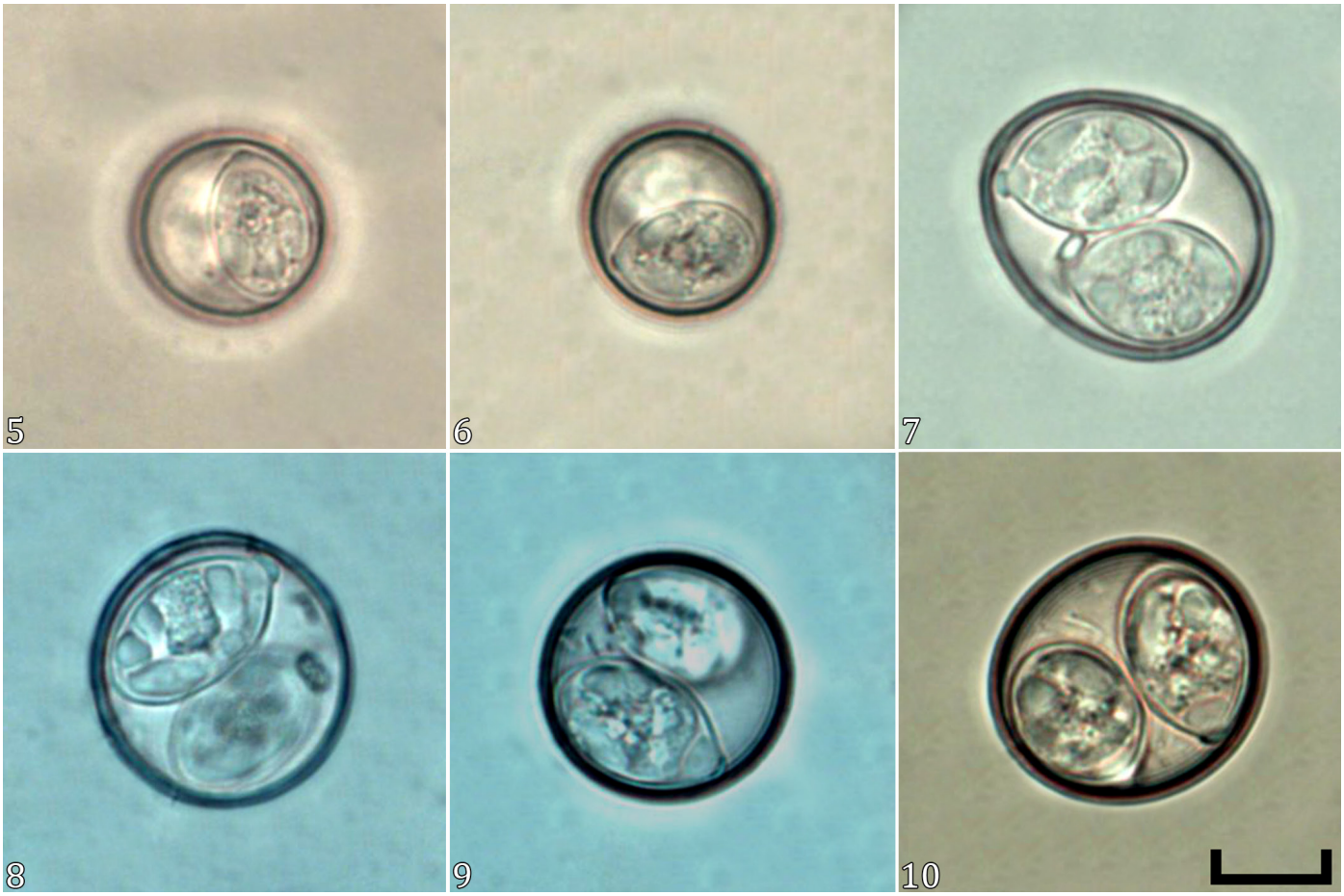

Fig.5-10. (**5-6**) Photomicrographs of sporulated oocysts of *Isospora saltatori*, (**7-8**) *Isospora trincaferri* and (**9-10**) *Isospora similisi* associated with clinical signs of coccidiosis in a free-living green-winged saltator *Saltator similis* in "Parque Nacional de Itatiaia" in southeastern Brazil. Bar = 10μm.

agent. Coelho et al. (2012) diagnosed coccidiosis in *S. similis* when evaluating different treatments for isosporosis in green-winged saltators rescued from illegal wildlife trafficking and kept in quarantine in CETAS/Seropédica. Vasconcellos et al. (2013) report coccidiosis associated with diarrhea and a high density of unidentified oocysts in green-winged saltators bred in captivity for the purpose of participating in bird singing competitions. All these studies involved captive birds; therefore, this seems to be the first report of coccidiosis in a free-living green-winged saltator.

Another point of discussion is the fact that the current study reports coccidiosis in a free-living bird caused by co-infection of the same three *Isospora* spp. recently characterized by Maronezi et al. (2022) in green-winged saltators living in captivity near "Parque Nacional de Itatiaia". In this context, as Maronezi et al. (2022) pointed out, keeping wild birds in captivity near conservation units represents an environmental risk to wildlife for several reasons. (1) Free-living birds can be infected by approaching and fighting with captive ones, mainly due to the territorial competition represented by their birdsong, which can reach long distances. (2) Captive birds, with potentially higher parasite densities (OPD), presumably provide a higher infective dose and would, therefore, increase the parasite density of free-living birds, causing ecological imbalance and possible epizootics. (3) According to the concept of parasite specificity of *Isospora* spp. at the host family level (Berto et al. 2011), any other bird of the family Thraupidae (or at least of the subfamily Saltatorinae) would be susceptible to infection transmitted by captive green-winged saltators, expanding the distribution and dispersion of these *Isospora* spp. in natural and conserved environments, and even placing pressure on endangered tanagers (Berto & Lopes 2020). Coelho et al. (2013) also state that it is essential to estimate coccidian density to avoid impacts of parasitism on natural bird populations, especially before the reintroduction/release of birds rescued from illegal wildlife trafficking. Similarly, Lopes et al. (2013) highlighted the role of illegal wildlife trafficking and reintroduction/release by wildlife rehabilitation centers in the dispersal of coccidians, as oocysts of *I. trincaferri* have already been identified in buff-throated saltators *Saltator maximus* (Müller, 1776) rescued from wildlife trafficking and kept in quarantine at CETAS/Seropédica.

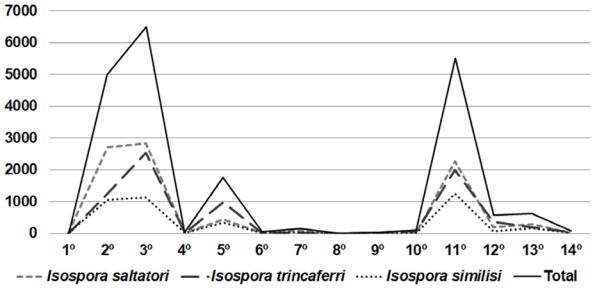

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Fig.11. Fluctuation of the total and specific density of *Isospora* spp. over two hours (between 4 and 6 p.m.) of sequential collection of fecal droplets from a free-living green-winged saltator *Saltator similis* with clinical signs of coccidiosis in "Parque Nacional de Itatiaia" in southeastern Brazil.

Finally, the current study identified morphologically three *Isospora* spp., probably the most commonly observed from *Saltator* spp. (Maronezi et al. 2022). However, molecular and/or serological methods should complement the characterization of these species in addition to the *locus* of the *cox1* gene of mitochondrial DNA provided by Maronezi et al. (2022), also characterizing whether or which of these *Isospora* spp. undergo an extraintestinal cycle, producing chronic and severe disease in passerines (Berto et al. 2023).

CONCLUSION

This study seems to be the first to report clinical signs of coccidiosis associated with a large number of oocysts of *Isospora* spp. in fecal samples from free-living green-winged saltators in "Parque Nacional de Itatiaia" (Itatiaia National Park), relating this finding to the recent discovery of *Isospora* spp. of green-winged saltators living in captivity near the park. Thus, this study emphasizes the importance of continuously evaluating birds' health as bioindicators and of their coccidian ecological biomarkers as one of the strategies for evaluating the conservation status of "Parque Nacional de Itatiaia".

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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