Braz. J. Vet. Parasitol., Jaboticabal, v. 26, n. 4, p. 457-464, oct.-dec. 2017 ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic) Doi: http://dx.doi.org/10.1590/S1984-29612017063

Diversity and distribution of coccidia of wild birds in an Atlantic forest fragment area in southeastern Brazil

Diversidade e distribuição de coccídios de aves silvestres em uma área de fragmento de Mata Atlântica no sudeste do Brasil

Patrícia Silva de Oliveira¹; Matheus Alexandre Ferreira²; Lidiane Maria da Silva¹; Mariana Borges Rodrigues¹; Sergian Vianna Cardozo³; Bruno Pereira Berto^{4*}

- ¹ Programa de Pós-graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro UFRRJ, Campus Seropédica, Seropédica, RJ, Brasil
- ² Escola de Ciências da Saúde, Universidade do Grande Rio UNIGRANRIO, Duque de Caxias, RJ, Brasil
- ³ Programa de Pós-graduação em Biomedicina Translacional, Universidade do Grande Rio UNIGRANRIO, Duque de Caxias, RJ, Brasil
- ⁴ Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro UFRRJ, Campus Seropédica, Seropédica, RJ, Brasil

Received August 16, 2017 Accepted October 9, 2017

Abstract

Coccidia are protozoan parasites that are frequently observed in fecal samples from wild birds, and they are extremely important for biodiversity, host specificity and conservation. The aim of the present study was to identify and quantify the coccidian species from wild birds caught in a fragmented area of Atlantic Forest in the municipality of Guapimirim in the state of Rio de Janeiro, which is located around the Serra dos Órgãos National Park. A total of 101 birds were caught and identified. The highest prevalence and density were observed in the family Columbidae (Columbiformes). Among the families of Passeriformes, the highest prevalences and densities were of birds in the families Thraupidae and Turdidae. The majority of the positive samples and those with higher densities were collected in the afternoons. Eleven coccidian species of *Isospora* and *Eimeria* were identified. Seven of these species that were morphologically identified are undescribed in the scientific literature and are believed to be new species. The present study highlights the wide distribution and dispersion of coccidia of wild birds in southeastern Brazil, and records the municipality of Guapimirim, in the state of Rio de Janeiro, as a new locality for parasitism, along with the new hosts recorded.

Keywords: Oocysts, morphology, taxonomy, prevalence, density, Parque Nacional da Serra dos Órgãos.

Resumo

Os coccídios são protozoários parasitas frequentemente observados em amostras fecais de aves silvestres, os quais têm extrema importância para a biodiversidade, especificidade hospedeira e conservação. O presente estudo teve como objetivo identificar e quantificar as espécies de coccídios de aves silvestres capturadas em uma área fragmentada de Mata Atlântica no município de Guapimirim, Estado do Rio de Janeiro, que está localizada no entorno do Parque Nacional da Serra dos Órgãos. Foram capturadas e identificadas 101 aves. A maior prevalência e densidades foram observadas na família Columbidae (Columbiformes). Nas famílias de Passeriformes, as maiores prevalências e densidades foram de aves das famílias Thraupidae e Turdidae. A maioria das amostras positivas e com maiores densidades foram coletadas no período da tarde. Foram identificadas onze espécies de *Eimeria e Isospora*. Sete dessas espécies morfologicamente identificadas não estão descritas na literatura científica e devem ser novas espécies. O presente trabalho expõe a ampla distribuição e dispersão de coccídios de aves silvestres no sudeste do Brasil e registra o município de Guapimirim, no estado do Rio de Janeiro, como uma nova localidade de parasitismo, além dos novos hospedeiros registrados.

Palavras-chave: Oocistos, morfologia, taxonomia, prevalência, densidade, Parque Nacional da Serra dos Órgáos.

^{*}Corresponding author: Bruno Pereira Berto. Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro – UFRRJ, Rodovia BR-465, Km 7, CEP 23897-000, Seropédica, RJ, Brasil. e-mail: berto.ufrrj@gmail.com

Introduction

Biodiversity refers to the variety of life on the planet, or to the distinctiveness of ecosystems. Brazil has a prominent position among the countries with the highest biodiversity because it hosts some of the biomes with the highest richness of animal species and also with the highest rates of endemism. In particular, the Atlantic Forest is among the top five in the list of global hotspots, since its remaining area is less than 8% of its original extent (SIMON et al., 2008).

In these remnant areas of Atlantic Forest, conservation units play a prominent role in maintaining the habitats and ecological niches of the species involved. Among the several Brazilian conservation units, the Serra dos Órgãos National Park (Parque Nacional da Serra dos Órgãos, PARNASO) stands out. This is located in the coastal mountain range (Serra do Mar) in the Serrana region of the state of Rio de Janeiro, within parts of the municipalities of Teresópolis, Petrópolis, Magé and Guapimirim. PARNASO hosts more than 2,800 plant species, 462 bird species, 105 mammal species, 103 amphibian species and 83 reptile species, including 130 endangered species and many endemic species (ICMBIO, 2017).

In general, the areas around these conservation units are fragmented or have been completely deforested for livestock, agricultural or urbanization activities, thus predisposing these areas to the edge effect. This effect consists of a change in the structure, composition or relative abundance of species at the edge of a forest. The edge effect caused by forest loss and fragmentation threatens the biodiversity of the Atlantic Forest, in addition to the direct impacts on fauna, flora and microbiota, which favor transmission of parasites to wild animals and susceptibility to parasites among wild animals. From this, it can be seen that knowledge about the parasites of wild birds is important, especially regarding some groups of parasites that have been little studied, such as coccidia (Apicomplexa: Eucoccidiorida), which are extremely important for biodiversity, host specificity and conservation (BERTO & LOPES, 2013).

Some scientific studies have been carried out on the ecology of coccidia of wild birds, mainly correlating the diversity and distribution of the coccidia with the feeding habits, biotopes, families and species of the host, sampling period, seasonality, urbanization of the environment, etc. (LÓPEZ et al., 2007; DOLNIK et al., 2010; MORIN-ADELINE et al., 2011; GIRAUDEAU et al., 2014). However, studies in this research area is very scarce in Brazil, despite the great biodiversity of birds and protected areas in the country.

In this context, the aim of the present study was to identify and quantify the coccidian species from wild birds caught in a fragmented area of Atlantic Forest in the municipality of Guapimirim, Rio de Janeiro, which is located around PARNASO.

Materials and Methods

A total of 13 expeditions were conducted in an area of Atlantic Forest in the municipality of Guapimirim, state of Rio de Janeiro, near PARNASO, but outside its limits (22°31'10" S; 43°00'36" W).

This area is occupied by a condominium of country houses called "Parque das Águas", which is located near the Rio-Teresópolis highway, at Km 1. Although the occupation of this condominium characterizes this area as urbanized, there are many conserved fragments of vegetation consisting of native Atlantic Forest.

Mist nets were set in the understory (up to 2.6m above the ground), opened at morning (6 AM-12AM) and afternoon (1 PM-6PM) and checked every 15 minutes. A total of 101 wild birds of different orders and families were caught (Table 1). The birds birds were immediately removed from the net and then kept in individual boxes and feces were collected immediately after defecation. After identification of the species, the birds were photographed and released.

The stool samples were placed in centrifuge tubes containing 2.5% potassium dichromate solution ($K_2Cr_2O_7$) at 1:6 (v/v). They were taken to the Laboratório de Biologia de Coccídios, Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ), where they were incubated at room temperature for one week or until ~70% of the oocysts were sporulated.

Oocysts were isolated by means of flotation in Sheather's saturated sugar solution (specific gravity, 1.20) and were examined under an optical microscope (DUSZYNSKI & WILBER, 1997). The oocysts were quantified as estimates of the number of oocysts per fecal drop (OPD), for which the total number of oocysts recovered from defecation was counted on a microscope slide, in accordance with the guidelines of Dolnik (2006). The oocysts were identified at 1000x magnification based on the guidelines for morphological and morphometric studies of Duszynski & Wilber (1997) and Berto et al. (2014b).

The ratio of positive samples to negative samples and the OPD respectively indicated the prevalence and density of coccidia (BUSH et al., 1997). Morphological observations, photomicrographs and measurements were made using an Olympus BX41 binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). All measurements are in micrometers and are given as the range followed by the mean in parentheses.

Comparative analyses were evaluated through the odds ratio test and Mann-Whitney test at the 5% significance level. All of these statistical tests were performed using the Bioestat 5.0 statistical software. Field-collecting permits were issued to B.P. Berto by SISBIO/ICMBio (license 42798-1) and CEUA/IV/UFRRJ (protocols 036/2014 and 6606250616).

Results

Distribution of coccidia in families

Birds of three orders and 14 distinct families were caught and identified. As expected, the order Passeriformes was the most representative in terms of the numbers of species (80%; 24/30) and specimens (88%; 89/101). The family with the greatest diversity of species caught was the Thraupidae (27%; 8/30), followed by the families Tyrannidae (17%; 5/30) and Turdidae (13%; 4/30). Thraupidae (34%; 34/101) was also the most representative family

Table 1. Prevalence and densities of coccidian parasites of the genera *Eimeria* or *Isospora* from wild birds captured in an Atlantic Forest fragmented area at Guapimirim, RJ, organized by order, family and species.

	Eimeria or Isospora Positive* Negative						
Orders/ Families/ Species		Positive*			— Total		
	AM**	PM	AM	PM			
Columbiformes: Columbidae							
Columbina talpacoti (Temminck, 1811)	0	1 (41)	0	1	2		
Leptotila verreauxi Bonaparte, 1855	1 (597)	4 (4759±5429)	1	0	6		
Subtotals:	1 (597)	5 (3815±5153)	1	2	8		
Passeriformes: Dendrocolaptidae							
Xiphorhynchus fuscus (Vieillot, 1818)	0	0	0	1	1		
Subtotals:	0	0	0	1	1		
Passeriformes: Fringillidae							
Euphonia violacea (Linnaeus, 1758)	0	0	1	0	1		
Subtotals:	0	0	1	0	1		
Passeriformes: Furnariidae							
Philydor atricapillus (Wied, 1821)	0	1 (74)	0	0	1		
Subtotals:	0	1 (74)	0	0	1		
Passeriformes: Hirundinidae							
Stelgidopteryx ruficollis (Vieillot, 1817)	0	0	0	1	1		
Subtotals:	0	0	0	1	1		
Passeriformes: Pipridae							
Manacus manacus Linnaeus, 1766	0	0	11	10	21		
Subtotals:	0	0	11	10	21		
Passeriformes: Thamnophilidae							
Dysithamnus mentalis (Temminck, 1823)	0	0	1	0	1		
Subtotals:	0	0	1	0	1		
Passeriformes: Thraupidae							
Coereba flaveola Linnaeus, 1758	0	3 (300±463)	1	1	5		
Lanio melanops (Vieillot, 1818)	0	1 (237)	1	1	3		
Sicalis flaveola (Linnaeus, 1766)	0	2 (5±1)	2	0	4		
Sporophila caerulescens (Vieillot, 1823)	0	0	6	1	7		
Tachyphonus coronatus Vieillot, 1822	0	0	2	0	2		
Tangara cyanocephala (Muller, 1776)	1 (2)	0	0	1	2		
Tangara palmarum (Wied, 1823)	0	0	1	0	1		
Tangara seledon (Muller, 1776)	0	5 (1,118±1,589)	0	5	10		
Subtotals:	1 (2)	11 (612±1,139)	13	9	34		
Passeriformes: Troglodytidae	- (-)	11 (012=1,10))			0.1		
Troglodytes musculus Naumann, 1823	0	1 (194)	2	1	4		
Subtotals:	0	1 (194)	2	1	4		
Passeriformes: Turdidae	v	1 (1)1)	2	•	•		
Turdus amaurochalinus Cabanis, 1850	0	1 (57)	2	0	3		
Turdus rufiventris Vieillot, 1818	0	3 (437±648)	3	3	9		
Turdus flavipes Vieillot, 1818	0	0	0	1	1		
Turdus leucomelas Vieillot, 1818	1 (140)	1 (6)	1	0	3		
Subtotals:	1 (140)	5 (275±509)	6	4	16		
Passeriformes: Tyrannidae	1 (140)) (2/)±309)	U	4	10		
	0	0	0	1	1		
Fluvicola nengeta (Linnaeus, 1766)	0	0	0	1	1		
Mionectes oleagineus (Lichtenstein, 1823)	0	0	1	0	1		
Myiozetetes similis (Spix, 1825)	0	0	1	0	1 5		
Pitangus sulphuratus (Linnaeus, 1766)	0	0	2	3	5		
Tolmomyias sulphurescens (Spix, 1825) Subtotals:	0 0	0 0	1 5	0 4	1 9		

^{*}Number of positives followed by mean density and standard deviation in parentheses; **Period of fecal sample collection at morning (6AM-12AM) or afternoon (1PM-6PM).

Table 1. Continued...

	Eimeria or Isospora						
Orders/ Families/ Species	Positive*			Negative			
	AM**	PM	AM PM		– Total		
Piciformes: Bucconidae							
Malacoptila striata (Spix, 1824)	0	0	1	0	1		
Subtotals:	0	0	1	0	1		
Piciformes: Galbulidae							
Galbula ruficauda Cuvier, 1816	0	0	1	0	1		
Subtotals:	0	0	1	0	1		
Piciformes: Picidae							
Celeus flavescens (Gmelin, 1788)	0	0	0	1	1		
Veniliornis maculifrons (Spix, 1824)	0	0	1	0	1		
Subtotals:	0	0	1	1	2		
Total:	3 (202±342)	23 (1,199±2,735)	41	34	101		

^{*}Number of positives followed by mean density and standard deviation in parentheses; **Period of fecal sample collection at morning (6AM-12AM) or afternoon (1PM-6PM).

in terms of the number of specimens caught, this time followed by the families Turdidae (16%; 16/101) and Tyrannidae (9%; 9/101).

The total number of birds caught and identified, along with their prevalences according to order, family and species, are shown in Table 1. Among the 14 different families of birds to which the specimens that were caught and identified belonged, only those in the families Furnariidae, Thraupidae, Troglodytidae and Turdidae of the order Passeriformes and the family Columbidae of the order Columbiformes eliminated oocysts of the genera *Isospora* or *Eimeria*. The oocysts were initially non-sporulated but sporulated 2 to 4 days after collection. No bird of the order Piciformes was positive for coccidia.

The highest prevalence of coccidia was observed in the family Columbidae (75%; 6/8). In the families of Passeriformes, lower prevalences were observed, such that the prevalences of Thraupidae and Turdidae were 35% (12/34) and 38% (6/16), respectively.

Regarding the OPD, the positive samples with highest densities were observed from *Leptotila verreauxi* Bonaparte, 1855, from which samples with OPD of 11,695 and 6,465 were obtained. From Passeriformes, the highest counts were from two specimens of *Tangara seledon* (Müller, 1776) and one of *Turdus rufiventris* Vieillot, 1818, which shed samples with OPD of 3,664, 1,703 and 1,183 respectively.

The majority of the positive samples and those with higher OPD were collected in the afternoons (1 PM to 6 PM). Statistical evaluation confirmed that this higher prevalence in the afternoons was significant, such that the likelihood of positivity for coccidia in the afternoons was nine times higher than in the mornings (Table 2). In contrast, the highest densities observed in the afternoons were not significantly different from those in the mornings.

Columbidae

From the family Columbidae, two different species were caught: *Columbina talpacoti* (Temminck, 1811) and *L. verreauxi*. Both species were positive for *Eimeria*. One specimen of *C. talpacoti* that was caught shed oocysts with morphology identical to that

described for *Eimeria curvata* Adriano, Thyssen, Cordeiro, 2000. This species was originally described from specimens of *C. talpacoti* and *Columbina squammata* (Lesson, 1831) that were caught in the municipality of Junqueirópolis, in the west of the state of São Paulo, Brazil (ADRIANO et al., 2000).

The oocysts identified as *E. curvata* in this study (Figures 1A, B) were ellipsoidal with a bilayered wall. No micropyle and oocyst residuum, but one polar granule was present. The sporocysts were elongated. The Stieda body was knob-like and prominent and the sub-Stieda body was absent. Sporocyst residuum was present as dispersed granules. The sporozoites were vermiform with two refractile bodies. The morphometry of the oocysts of *E. curvata* is shown in Table 3.

Five specimens of *L. verreauxi* shed oocysts of *Eimeria*. However, no coccidia had previously been described parasitizing *Leptotila* spp. In addition, the morphology observed in these oocysts was not compatible with any species of *Eimeria* described from Columbiformes.

Furnariidae

From the family Furnariidae, only one bird was caught, but it was positive for Isospora. To date, only three species of Isospora have been described from Furnariidae, all in Ecuador: Isospora hyloctistum McQuistion, Capparella, 1994, from Automolus subulatus (Spix, 1824); Isospora scleruri McQuistion, Capparella, 1994, from Sclerurus spp.; and Isospora automoli McQuistion, Barber, Capparella, 1999, from Automolus spp. (MCQUISTION & CAPPARELLA, 1994; MCQUISTION et al., 1999; BERTO et al., 2011). Although these Isospora spp. have been described in the trans-Andean region, some studies have reported transmission across the Andes, mainly by birds inhabiting both trans- and cis-Andean regions (BERTO et al., 2014a; SILVA et al., 2017). Even so, the oocysts observed in the current study were morphologically incompatible with these three Isospora spp. described from New World furnariids. Therefore, this species should be considered to be a new species after further observations and a detailed taxonomic description.



Figure 1. Photomicrographs of sporulated oocysts of (A, B) *Eimeria curvata* from *Columbina talpacoti*; (C, D) *Isospora cagasebi* from *Coereba flaveola*; (E, F) *Isospora cetasiensis* from *Sicalis flaveola*; and (G, H) *Isospora massardi* from *Turdus* spp. in an area of Atlantic Forest in the municipality of Guapimirim, Rio de Janeiro, Brazil. Bar=10 µm.

Table 2. Prevalence and density of coccidian parasites of the genera *Eimeria* or *Isospora* from wild birds, in relation to the period of fecal sample collection, in an Atlantic Forest fragmented area at Guapimirim in southeastern Brazil.

	Coccidia (Eimeria or Isospora)						
Period	Prevalence*			Density**			
	+/N (%)	OR	95% CI	p-value	Mean	SD	p-value
Morning (6AM-12AM)	3/44 (6.8%)	Ref.			202	342	0.179
Afternoon (1PM-6PM)	23/57 (40.4%)	9.2451	2.5547-33.4572	0.0003	1199	2735	

Statistical comparisons made by the Odds Ratio* and Mann-Whitney test**. +: number of positive animals; N: number of samples; OR: odds ratio; 95% CI: 95% confidence interval; Ref.: variable used as a reference value; SD: standard deviation.

Table 3. Morphometry of coccidian parasites of the genera *Eimeria* or *Isospora* from wild birds in an Atlantic Forest fragmented area at Guapimirim in southeastern Brazil.

		Oocyst			Sporocyst			
Species	Host	Length (µm)	Width (µm)	Shape index	Length (µm)	Width (µm)	Shape index	
Eimeria curvata Adriano, Thyssen, Cordeiro, 2000	Columbina talpacoti (Temminck, 1811) (Columbiformes: Columbidae)	17.7 (16-19)	14.9 (13-16)	1.2 (1.1-1.5)	11.8 (11-12)	5.7 (5-6)	2.1 (2.0-2.2)	
Isospora cagasebi Berto, Flausino, Luz, Ferreira, Lopes, 2008	Coereba flaveola Linnaeus, 1758 (Passeriformes: Thraupidae)	27.7 (26-29)	26.5 (24-27)	1.0 (1.0-1.1)	19.3 (19-20)	11.8 (11-13)	1.6 (1.5-1.7)	
Isospora cetasiensis Coelho, Berto, Neves, Oliveira, Flausino, Lopes, 2011	Sicalis flaveola (Linnaeus, 1766) (Passeriformes: Thraupidae)	25.3 (24-26)	23.1 (23-24)	1.1 (1.0-1.1)	16.2 (16-17)	11.2 (11-12)	1.4 (1.4-1.5)	
Isospora massardi Lopes, Berto, Luz, Galvão, Ferreira, Lopes, 2014	Turdus albicollis Vieillot, 1818; Turdus amaurochalinus Cabanis, 1850; Turdus rufiventris Vieillot, 1818; Turdus leucomelas Vieillot, 1818 (Passeriformes: Turdidae)	19.1 (18-20)	18.1 (17-19)	1.1 (1.0-1.1)	14.7 (13-15)	8.5 (8-9)	1.7 (1.6-1.9)	

Thraupidae

From the family Thraupidae, five bird species were positive. Three specimens of *Coereba flaveola* Linnaeus, 1758, that were caught shed oocysts with morphology identical to that described for *Isospora cagasebi* Berto, Flausino, Luz, Ferreira, Lopes, 2008. This species was originally described from *C. flaveola* on Marambaia island, in southeastern Brazil (BERTO et al., 2008, 2011).

The oocysts identified as *I. cagasebi* in this study (Figures 1C, D) were sub-spherical with a bilayered wall. No micropyle, polar granule or oocyst residuum. The sporocysts were elongate ovoidal. The Stieda body was knob-like and the sub-Stieda body was rounded. Sporocyst residuum was present as dispersed granules. The sporozoites were vermiform with one refractile body and a nucleus. The morphometry of the oocysts of *I. cagasebi* is shown in Table 3.

One of the three specimens of *Lanio melanops* (Vieillot, 1818) that was caught was positive for *Isospora* sp. However, this coccidian species could not be identified through morphological comparison with the other species of Thraupidae that were described (BERTO et al., 2011). In addition, the present study provides the first report of *Isospora* sp. from *L. melanops*.

Half of the saffron finches *Sicalis flaveola* (Linnaeus, 1766) that were caught were positive for a coccidian species identified as *Isospora cetasiensis* Coelho, Berto, Neves, Oliveira, Flausino, Lopes, 2011. *Sicalis flaveola* is a very common bird species in Brazil and the original description of *I. cetasiensis* was from saffron finches that had been recovered from illegal trade and were being kept at the Center for Wild Animal Screening (Centro de Triagem de Animais Silvestres, CETAS) of the municipality of Seropédica, in the state of Rio de Janeiro, for rehabilitation and reintroduction into the wild (COELHO et al., 2011).

The oocysts identified as *I. cetasiensis* in this study (Figures 1E, F) were subspherical to ellipsoidal with a bilayered wall. No micropyle, polar granule or oocyst residuum. The sporocysts were ovoidal. The Stieda body was knob-like and the sub-Stieda body was rounded. Sporocyst residuum was present as dispersed granules and spherules of different sizes. The sporozoites were vermiform with one refractile body and a nucleus. The morphometry of the oocysts of *I. cetasiensis* is shown in Table 3.

The tanagers *Tangara cyanocephala* (Müller, 1776) and *T. seledon* were positive for three *Isospora* spp. However, none of these were compatible with the morphologies recorded for the other coccidian species described from Thraupidae. Although some *Isospora* spp. have been described from *Tangara* spp., to date there are no reports of *Isospora* spp. from *T. cyanocephala* and *T. seledon* specifically (BERTO et al., 2011).

Troglodytidae

From the family Troglodytidae, one of the four specimens of *Troglodytes musculus* Naumann, 1823, that was caught was positive for *Isospora* sp. Recently, the species *Isospora corruirae* Lopes, Rodrigues, Silva, Berto, Luz, Ferreira, Lopes, 2016, was described infecting *T. musculus* on Marambaia island, southeastern Brazil (LOPES et al., 2016). However, the oocysts recovered

from *T. musculus* in the current study could not be identified as belonging to this species.

Turdidae

Six of the sixteen thrushes of the family Turdidae that were caught were positive for *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira, Lopes, 2014. This species was originally described infecting *Turdus albicollis* Vieillot, 1818, on Marambaia island, southeastern Brazil (LOPES et al., 2014). In this regard, the thrushes *Turdus amaurochalinus* Cabanis, 1850, *Turdus rufiventris* Vieillot, 1818, and *Turdus leucomelas* Vieillot, 1818, which were positive for *I. massardi* in the current study, become new hosts for this coccidian species.

The oocysts identified as *I. massardi* in this study (Figures 1G, H) were subspherical with a bilayered wall. No micropyle and oocyst residuum, but frequently two polar granules were present. The sporocysts were ovoidal. The Stieda body was knob-like to rounded and the sub-Stieda body was rounded. Sporocyst residuum was present as dispersed spherules of different sizes. The sporozoites were vermiform with posterior and anterior refractile bodies and a nucleus. The morphometry of the oocysts of *I. massardi* is shown in Table 3.

Discussion

In the current study the overwhelming majority of birds belonged to the Thraupidae family. This greater presence of thraupids in this area of Atlantic Forest in Guapimirim may be associated with their feeding habit, which is predominantly frugivorous (SICK, 1997). The area studied has many fruit trees that have been planted, and many residents offer fruit in the yards of their homes and on the edge of the forest.

The families with highest prevalences and densities of coccidia have the common feature of feeding habits that are omnivorous, frugivorous and/or granivorous. Coccidia of *Eimeria* and *Isospora* have fecal-oral transmission and, in this regard, the habit of feeding and defecating in the same locality, which is characteristic of these birds, should favor transmission and consequently higher prevalence and densities in these families. On the other hand, families of predominantly insectivorous and/or carnivorous birds were negative for coccidia or had low prevalence and density (DOLNIK et al., 2010).

Statistical analyses of the prevalences and densities of coccidia could not be evaluated by variables such as host species or families due to the low number of positives in most families/species; however, the values clearly show the highest prevalences and densities in the families Columbidae and Thraupidae, which are families of birds omnivorous, frugivorous and/or granivorous (Table 1). On the other hand, period of fecal sample collection provided statistically significant differences for prevalence and the lack of significance between the densities of samples collected in the mornings and afternoons should be related to the low number of positive samples collected in the mornings (Table 2).

These observations are in accordance with the findings of López et al. (2007), Martinaud et al. (2009), Dolnik et al. (2010)

and Morin-Adeline et al. (2011), who reported that the coccidia birds have a circadian rhythm of oocyst shedding, tending to shed more oocysts in the of last hours of the day. So far, these shedding dynamics have been correlated with the advantages of transmission during the peak of feeding activity during the afternoons and with protection from solar UV radiation (LÓPEZ et al., 2007; MARTINAUD et al., 2009).

It was observed that the birds that were caught seemed to be healthy, that is, they did not have the clinical signs often involved with coccidiosis, such as cachexia, rufled feathers, diarrhea, etc., including those parasitized by coccidia. This information allied with the low prevalence and densities of coccidia in wild birds become important from the point of view of nature conservation in the study area, as it is demonstrated that in disturbed areas there is a strong correlation with severe coccidiosis (GIRAUDEAU et al., 2014). In this way, it can be concluded that despite the partial anthropization of the study area, wild birds have not been adversely impacted.

Finally, the identification here of coccidian species that are unreported in the scientific literature emphasizes how little is known about the diversity and distribution of coccidia in wild birds. These undescribed species reported in the current study should be considered to be as new species after further observations and detailed taxonomic descriptions in future publications. In addition, identification of these coccidian species that were originally reported in distinct locations far away from the location of the present study demonstrates the wide distribution and dispersion of coccidia of wild birds in southeastern Brazil. Thus, the municipality of Guapimirim, in the state of Rio de Janeiro, is recorded as a new locality of parasitism, in addition to the cases cited, in which new hosts were recorded.

Acknowledgements

We thank the owner of a country house in the condominium "Parque das Águas" in the municipality of Guapimirim, in the state of Rio de Janeiro, who allowed us to access and use some facilities during the expeditions. This study was supported by grants from the Coordination Office for Improvement of Higher-Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES), Carlos Chagas Filho Research Support Foundation of the State of Rio de Janeiro (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, FAPERJ), National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq) and National Foundação Nacional de Desenvolvimento do Ensino Superior Particular, FUNADESP).

References

Adriano EA, Thyssen PJ, Cordeiro NS. *Eimeria curvata* n. sp. (Apicomplexa: Eimeriidae) in *Columbina talpacoti* and *Scardafella squammata* (Aves: Columbidae) from Brazil. *Mem Inst Oswaldo Cruz* 2000; 95(1): 53-55. PMid:10656705. http://dx.doi.org/10.1590/S0074-02762000000100008.

Berto BP, Flausino W, Luz HR, Ferreira I, Lopes CWG. *Isospora cagasebi* sp. nov. (Apicomplexa, Eimeriidae) from the bananaquit, *Coereba flaveola* of Brazil. *Acta Parasitol* 2008; 53(2): 117-119. http://dx.doi.org/10.2478/s11686-008-0025-8.

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). *Syst Parasitol* 2011; 80(3): 159-204. PMid:22002022. http://dx.doi.org/10.1007/s11230-011-9317-8.

Berto BP, Lopes CWG. Distribution and dispersion of coccidia in wild passerines of the Americas. In: Ruiz L, Iglesias L, editors. *Birds: evolution and behavior, breeding strategies, migration and spread of disease*. New York: Nova Science Publishers; 2013. p. 47-66.

Berto BP, Lopes BB, Melinski RD, Souza AHN, Ribas CC, Abreu FH, et al. Coccidial dispersion across *trans-* and *cis-*Andean antbirds (Passeriformes: Thamnophilidae): *Isospora sagittulae* (Apicomplexa: Eimeriidae) from nonsympatric hosts. *Can J Zool* 2014a; 92(5): 383-388. http://dx.doi.org/10.1139/cjz-2013-0277.

Berto BP, McIntosh D, Lopes CWG. Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Rev Bras Parasitol Vet* 2014b; 23(1): 1-15. PMid:24728354. http://dx.doi.org/10.1590/S1984-29612014001.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83(4): 575-583. PMid:9267395. http://dx.doi.org/10.2307/3284227.

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 Parasitol* 2011; 56(3): 239-244. http://dx.doi.org/10.2478/s11686-011-0051-9.

Dolnik O. The relative stability of chronic *Isospora sylvianthina* (Protozoa: Apicomplexa) infection in Blackcaps (*Sylvia atricapilla*): evaluation of a simplified method of estimating isosporan infection intensity in passerine birds. *Parasitol Res* 2006; 100(1): 155-160. PMid:16874477. http://dx.doi.org/10.1007/s00436-006-0253-5.

Dolnik OV, Dolnik VR, Bairlein F. The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. *Ardea* 2010; 98(1): 97-103. http://dx.doi.org/10.5253/078.098.0112.

Duszynski DW, Wilber PG. A guideline for the preparation of species descriptions in the Eimeriidae. *J Parasitol* 1997; 83(2): 333-336. PMid:9105325. http://dx.doi.org/10.2307/3284470.

Giraudeau M, Mousel M, Earl S, McGraw K. Parasites in the city: degree of urbanization predicts poxvirus and coccidian infections in house finches (*Haemorhous mexicanus*). *PLoS One* 2014; 9(2): e86747. PMid:24503816. http://dx.doi.org/10.1371/journal.pone.0086747.

Lopes BB, Berto BP, Luz HR, Galvão GS, Ferreira I, Lopes CWG. *Isospora massardi* sp. nov. (Apicomplexa: Eimeriidae) from the whitenecked thrush *Turdus albicollis* (Passeriformes: Turdidae) from Brazil. *Acta Parasitol* 2014; 59(2): 272-275. PMid:24827098. http://dx.doi.org/10.2478/s11686-014-0240-4.

Lopes BB, Rodrigues MB, Silva LM, Berto BP, Luz HR, Ferreira I, et al. A new isosporoid coccidia (Apicomplexa: Eimeriidae) from the southern house wren *Troglodytes musculus* Naumann, 1823 (Passeriformes: Troglodytidae) from Brazil. *Acta Parasitol* 2016; 61(2): 425-428. http://dx.doi.org/10.1515/ap-2016-0057. PMid:27078670.

López G, Figuerola J, Soriguer R. Time of day, age and feeding habits influence coccidian oocyst shedding in wild passerines. *Int J Parasitol*

2007; 37(5): 559-564. PMid:17289051. http://dx.doi.org/10.1016/j. ijpara.2006.12.014.

Martinaud G, Billaudelle M, Moreau J. Circadian variation in shedding of the oocysts of *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): an adaptative trait against desiccation and ultraviolet radiation. *Int J Parasitol* 2009; 39(6): 735-739. PMid:19100268. http://dx.doi.org/10.1016/j.ijpara.2008.11.006.

McQuistion TE, Barber CY, Capparella AP. *Isospora automoli*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the buff-throated foliage-gleaner *Automolus ochrolaemus* and the olive-backed foliage gleaner *A. infuscatus* from South America. *Syst Parasitol* 1999; 44(1): 71-73. http://dx.doi.org/10.1023/A:1006121923716.

McQuistion TE, Capparella A. Two new species of *Isospora* (Apicomplexa: Eimeriidae) from ovenbirds (Passeriformes: Furnariidae) of South America. *Trans Am Microsc Soc* 1994; 113(1): 90-95. http://dx.doi.org/10.2307/3226584.

Morin-Adeline V, Vogelnest L, Dhand NK, Shiels M, Angus W, Slapeta J. Afternoon shedding of a new species of *Isospora* (Apicomplexa) in

the endangered Regent Honeyeater (*Xanthomyza phrygia*). *Parasitology* 2011; 138(6): 713-724. PMid:24650932. http://dx.doi.org/10.1017/S0031182011000126.

Parque Nacional Serra dos Órgãos – ICMBIO. [online]. Teresópolis: ICMBIO; 2017 [cited 2017 July 10]. Available from: http://www.icmbio.gov.br/parnaserradosorgaos

Sick H. Ornitologia brasileira. Brasília: Nova Fronteira; 1997.

Silva LM, Rodrigues MB, Pinho IF, Lopes BB, Luz HR, Ferreira I, et al. Some remarks on the distribution and dispersion of Coccidia from icterid birds in South America: *Isospora guaxi* n. sp. and *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 (Apicomplexa: Eimeriidae) from the redrumped cacique *Cacicus haemorrhous* (L.) (Passeriformes: Icteridae) in southeastern Brazil. *Syst Parasitol* 2017; 94(1): 151-157. PMid:28062994. http://dx.doi.org/10.1007/s11230-016-9688-y.

Simon JE, Peres J, Ruschi PA. A importância da Serra das Torres para a conservação de aves no estado do Espírito Santo, Sudeste do Brasil. *Rev Cient FAESA* 2008; 4(1): 47-62. http://dx.doi.org/10.5008/1809-7367.017.