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Abstract. *Isospora sepetibensis* Berto, Flausino, Luz, Ferreira and Lopes, 2008 is a protozoan coccidian parasite (Chromista: Miozoa: Coccidiomorpha: Coccidia) that was originally described from Brazilian tanagers *Ramphocelus bresilius* (Linnaeus, 1766) in the Marambaia Island in the Coast of the State of Rio de Janeiro. In the current work, this species was identified from black-goggled tanagers *Trichothraupis melanops* (Vieillot, 1818) in the Itatiaia National Park, which is a protected area with a high degree of vulnerability in the interior of the State of Rio de Janeiro, distant in more than 100 km of the type-locality. Its oocysts are sub-spherical to elongate ovoidal, 25.9 × 20.7 µm with smooth, bi-layered wall, ~ 1.3 µm and length/width ratio of 1.1–1.4 (1.26). Micropyle and oocyst residuum absent, but one or two polar granules are present. Sporocysts are ellipsoidal, 16.8 × 10.3 µm, with both Stieda and sub-Stieda bodies. Sporocyst residuum present and sporozoites with refractile body and nucleus. Molecular analysis was conducted at the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene. This new isolate exhibited similarity greater than 98% with *Isospora* spp. isolates from other Neotropical passerines and with an *Isospora* sp. pseudoparasite of voles of Eurasia. This is the first coccidian parasite from a New World tanager to have a molecular identification of the *cox1* gene.

Key words: morphology, molecular biology, taxonomy, phylogeny, Coccidia, oocysts, Neotropical birds, Thraupidae, Parque Nacional do Itatiaia.

INTRODUCTION

The subclass Coccidia (Chromista: Miozoa: Coccidiomorpha) brings together various genera of parasites of all classes of vertebrates, which may be associated with enteritis and death (Fayer 1980; Ruggiero et al. 2015). In Passeriformes, the main genera are *Isospora* Schneider, 1881 and, infrequently, *Eimeria* Schneider, 1875 (Berto et al. 2011a).

The black-goggled tanager *Trichothraupis melanops* (Vieillot, 1818) is characterized by presenting the yellowish-colored pile contrasting with a black face.
The final edited and typeset version of record will appear in future Accepted, unedited articles published online and citable. The final edited and typeset version of record will appear in future

MATERIAL AND METHODS

Sample collection: Four expeditions were conducted in the Itatiaia National Park (Parque Nacional do Itatiaia), a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira (ICMBIO 2019). Sampling occurred in April 2017 (22°26′17.00″S, 44°37′33.00″W and 22°27′20.58″S, 44°36′28.58″O), June 2017 (22°27′4.00″S, 44°36′51.00″O) and July 2017 (22°26′17.00″S, 44°37′33.00″O). A total of fifteen T. melanops were captured with mist nets. The birds were kept in individual boxes and feces collected immediately after defection. After identification of the species, the birds were photographed and released and stool samples were placed in centrifuge tubes containing a potassium dichromate 2.5% (K₂Cr₂O₇) solution at 1:6 (v/v).

Morphological analyses: Samples were taken to the Laboratório de Biologia de Coccídisios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature (25°C) for 10 days or until ~70% of the oocysts were sporulated. Oocysts were isolated by flotation in Sheather’s sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2008). In the current work, this coccidian species is identified from the new host T. melanops in the interior of the State of the Rio de Janeiro, providing a preliminary genotypic characterization via sequencing of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene.

Results

Fifteen black-goggled tanagers were examined and ten of them shed oocysts in the feces. All observed oocysts were morphologically identified as I. sepetibensis. This material is described below.

Isospora sepetibensis Berto, Flausino, Luz, Ferreira, Lopes, 2008 (Fig. 1)

Description of sporulated oocyst: Oocyst shape (n = 18) sub-spherical to elongate ovoidal; 22–29 × 19–22 (25.9 × 20.7) length/width (L/W) ratio 1.1–1.4 (1.26). Wall bi-layered, 1.1–2.0 (1.3) thick, outer layer smooth, c.2/3 of total thickness. Micropyle and oocyst residuum absent, but one or two polar granules are present.

Description of sporocyst and sporozoites: Sporocysts (n = 18), ellipsoidal, 15–18 × 9–11 (16.8 × 10.3);...
Fig. 1. Photomicrographs of sporulated oocysts of *Isospora sepetibensis*, a coccidium species recovered from the black-goggled tanager *Trichothraupis melanops*. Note the inner (il) and outer (ol) layer of the oocyst wall, nucleus (n), polar granule (pg), Stieda body (sb), sub-Stieda body (ssb), sporocyst residuum (sr), striations (str) and the refractile body (rb). Sheather’s sugar solution. Scale-bar: 10 µm.

L/W ratio 1.5–1.8 (1.65). Stieda body present, knob-like, 1.1–1.3 (1.2) high, 2.0–2.2 (2.1) wide; sub-Stieda body present, large to trapezoidal or, occasionally, irregular, 1.2–2.3 (1.7) high, 2.7–3.5 (3.1) wide; para-Stieda body absent. Sporocyst residuum present, consisting of numerous small granules dispersed between the sporozoites or as a distinctly sub-spherical body that appear to be membrane-bounded, 5.5–6.7 (6.1). Sporozoite vermiform with one posterior refractile body, centrally located nucleus and striations.

**Host:** *Trichothraupis melanops* (Vieillot, 1818) (Passeriformes: Thraupidae: Thraupinae).

**Locality:** Itatiaia National Park (22°26’17.00"S, 44°37’33.00"W), Southeastern Brazil.

**Specimens:** Photomicrographs and oocysts in 2.5% *K₂Cr₂O₇* solution (Williams *et al.* 2010) are deposited at the Museu de Zoologia at the Universidade Federal Rural do Rio de Janeiro, Brazil, under accession number MZRPTZ2019014. Photomicrographs are also deposited and available (http://r1.ufrj.br/labico/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 92/2019. Photographs of the host specimens are deposited in the same collection.

**Site in host:** Unknown.

**Prevalence:** 67% (15 out of 10 birds infected).

**Representative DNA sequence:** Representative *cox1* sequence is deposited in the GenBank database under the accession number MK682606.

**Phylogenetic analysis:** The amplification of the DNA from the fifteen oocysts of *I. sepetibensis* recovered from *T. melanops* showed a clear band of c. 250 bp. The phylogenetic analysis was constructed with 33 sequences of *Isospora* spp. closest to *I. sepetibensis*
available on GenBank (Fig. 2). *Eimeria tenella* (Railliet, Lucet, 1891) was used as the outgroup. *Isospora sepetibensis* sat in a large group with the highest similarity of 98.1% with an *Isospora* sp. reported as pseudoparasite of bank voles *Myodes glareolus* (Schreber, 1780) in Czech Republic (Trefancová et al. 2019). It was close to the clade with *Isospora* isolates from the spectacled warbler *Sylvia conspicillata* Temminck, 1820 in Macaronesia (Illera et al. 2015); and also was close to the clade with *Isospora* spp. recently sequenced from Neotropical passerines (Silva-Carvalho et al. 2018a; 2018b; Rodrigues et al. 2019). Subsequently, a subset with only 215 bp long *cox1* gene sequences was constructed (Fig. 3). In this analysis, *Isospora sepetibensis* sat in the same clade of *Isospora* spp. from Neotropical passerines, with the highest similarity of 98.5% with *Isospora lopesi* Silva-Carvalho & Berto, 2018 (Silva-Carvalho et al. 2018a).

**DISCUSSION**

The morphological data reported in the current work showed a high degree of similarity to the descriptions from the Brazilian tanager *R. brevipes* and from the blue dacnis *Dacnis cayana* (Linnaeus, 1766) (Table 1) (Berto et al. 2008; 2011b). Despite the polymorphism already characterized for this coccidian species (Berto...
et al. 2011b), patterns of shape/size may be related to each of the hosts and/or localities. The oocysts from *R. bresilius* were smaller and more rounded, as can be confirmed by the lower L/W ratio (Berto et al. 2014); whereas, the oocysts from *D. cayana* were smaller; and the oocysts from *T. melanops* were quite elongated and larger than those from *D. cayana* (Table 1). In addition, some specimens from *T. melanops* had an irregular sub-Stieda (Fig. 1c), which was not observed in the previous works (Berto et al. 2008; 2011b). Possibly, these patterns/differences may be associated to the process of speciation and adaptation to new hosts and/or localities, or other associated factors (Fayer 1980; Gardner and Duszynski 1990).

The new locality of parasitism of *I. sepetibensis* recorded in the current work is noteworthy. The Marambaia Island, where the two previous reports occurred, is more than 100 km away and more than 1,000 m of altitude in the Itatiaia National Park, on the opposite border of the State of Rio de Janeiro. This observation highlights the great dispersion of this coccidian species in the State of Rio de Janeiro and its adaptation to considerably different environments (Berto and Lopes 2013).

Traditionally, characterization of avian species of *Isospora* has mainly been based on morphological features and host specificity; however, this can be problematic whenever there are morphological equivalents from different hosts, environments or from localities that disfavor the transmissions (Gardner and Duszynski 1990; Berto et al. 2011a; Hafeez et al. 2014). In this sense, molecular studies become relevant. Molecular methods, as amplification and sequencing of specific genes, complement the morphological studies with new information about genetic diversity and can also address questions about phylogenetic relationships and phylogeographic, which are hard to answer only with morphological data (Perkins and Shall 2002; Adkesson et al. 2005; Schrenzel et al. 2005; Berto et al. 2011a).

In the last 10 years, molecular methods have increasingly been used in studies of diversity, distribution, specificity, ecology, and different aspects of evolutionary biology of avian parasites (Bensch et al. 2000; Waldenström et al. 2002). In this context, the *cox1* gene was chosen for genotyping in the current work because it has been indicated as the most suitable for phylogenetic studies by having a higher resolving power than the 18S rRNA gene in delineating recent speciation events (Yang et al. 2015; Ogedengbe et al. 2011; Silveira-Carvalho et al. 2018a).

*Isospora sepetibensis* is the first coccidian parasite of a New World tanager to have its *cox1* sequence deposited in the GenBank database. The phylogenetic proximity of this coccidian species to the pseudoparasite isolate from the bank voles in Czech Republic weakens the theory of the formation of a monophyletic
group with *Isospora* spp. from Neotropical passerines (Rodrigues et al. 2019; Trefancová et al. 2019). Although it is not known which is the true host of this *Isospora* sp. isolated from the feces of the bank voles, it is logically known that it is not a Neotropical passerine. Anyway, with the current frequency of sequences of *Isospora* spp. from passerines being deposited at GenBank, phylogenetic studies will be more complete and conclusive, especially with the 215 bp long *cox1* gene sequences that have already been considered more useful for specific phylogenetic studies (Yang et al. 2015; 2016). In this analysis, *I. sepetibensis* sat in a monophyletic group with *Isospora* spp. from Neotropical passerines, but greater inferences are not possible due to the low number of 215 bp long *cox1* gene sequences deposited in the GenBank database.

Finally, based on all the results reported in the current work, *T. melanops* is recorded as a new host for *I. sepetibensis* in the Itatiaia National Park in South-eastern Brazil; and, in addition, this is the first coccidian parasite from a New World tanager to have a molecular identification of the *cox1* gene.

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**REFERENCES**


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