

***Isospora brasilsatoae* n. sp. (Apicomplexa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) from Brazil**

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Abstract

A new species of coccidia (Protozoa: Apicomplexa: Eimeriidae) found parasitizing the yellow-legged thrush *Turdus flavipes* (Vieillot, 1818), the pale-breasted thrush *Turdus leucomelas* Vieillot, 1818 and the creamy-bellied thrush *Turdus amaurochalinus* Cabanis, 1850 in Southeastern Brazil is described. *Isospora brasilsatoae* n. sp. has oocysts that are ovoidal to pyriform, 26.8 × 21.1 µm, with smooth, bilayered wall, ~1.6 µm thick. Micropyle present. Oocyst residuum absent, but one to three polar granules are present. Sporocysts are elongate ovoidal, 18.1 × 10.5 µm. Stieda body knob-like and sub-Stieda body trapezoidal. Sporocyst residuum is composed of granules that appear to be membrane-bounded. Sporozoites are vermiform with refractile bodies and striations. This is the ninth description of an *Isospora* sp. parasitizing a New World thrush.

Key words: taxonomy, morphology, coccidia, *Isospora*, oocysts, Passeriformes, Turdidae, Itatiaia National Park, Serra dos Órgãos National Park, Guapimirim, Cacaria, Rio de Janeiro, Brazil

Introduction

Brazil has 14% of the biodiversity of the world. The explanation for this biological richness lies in the large number of endemic species, the enormous vegetation cover, the variety of biomes and the immense coastline (Lewinsohn & Prado 2005). Brazil occupies 47.8% of the Neotropical region, being known as the 'country of the birds' for having about a third of all species on the planet, representing almost 57% of the total resident birds of South America. In this sense, Brazil becomes one of the most important countries for investment in bird conservation in the world (Sick 1997; Lewinsohn & Prado 2005; Marini & Garcia 2005).

Among all families of Aves in Brazil, the Turdidae family comprises 20 genera and 180 species, of which 87 are classified in the genus *Turdus* Linnaeus, 1758. *Turdus* spp. are abundant in the Atlantic and Amazon forests, preferably inhabiting humid areas with higher trees on slopes, but also found near the soil feeding on small fruits and insects (Sick 1997; BirdLife International 2016).

Wild birds are hosts of endo- and ectoparasites; however, among all these, coccidian protozoa can be highlighted. Parasitism by coccidiens should not affect the health of wild birds in preserved/conserved environments; however, in impacted environments, stress and consequent low immunity make wild birds more susceptible to coccidiosis (Berto & Lopes 2013).

In this context, the current study describes a new species of *Isospora* recovered from yellow-legged thrushes

Turdus flavipes (Vieillot, 1818), pale-breasted thrushes *Turdus leucomelas* Vieillot, 1818 and creamy-bellied thrushes *Turdus amaurochalinus* Cabanis, 1850 captured in different localities in Southeastern Brazil.

Material and methods

A total of four expeditions were conducted in 3 different localities in southeastern Brazil: (1) Parque Nacional do Itatiaia ($22^{\circ}26'57"S$, $44^{\circ}36'25"W$), a protected area located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais and São Paulo (ICMBIO 2018a); (2) a fragmented area of Atlantic Forest in the Municipality of Guapimirim ($22^{\circ}31'10"S$, $43^{\circ}00'36"W$), which is located around Parque Nacional da Serra dos Órgãos, another protected area located in the coastal mountain range (Serra do Mar) of the State of Rio de Janeiro (ICMBIO 2018b); and (3) Cacaria ($22^{\circ}42'51"S$, $43^{\circ}50'38"W$) at the Municipality of Piraí in the State of the Rio de Janeiro. A total of five *T. flavipes* (four from Parque Nacional do Itatiaia and one from Cacaria), four *T. leucomelas* (two from Parque Nacional do Itatiaia and two from Guapimirim) and one *T. amaurochalinus* (from Cacaria) were captured with mist nets. The birds were kept in individual boxes with clean ground paper. After identification of the species (Sigrist 2014), the bird was photographed and released. A fresh droplet of feces from each individual bird was placed in an individually centrifuge tube with a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution. Field-collecting permits were issued to B.P. Berto by SISBIO/ICMBio (licenses 42798-1; 45200-1; 49605-1; 54951-1) and CEUA/UFRRJ (protocols IV-036/2014; ICBS-008/2015; IV-6606250616). Fecal samples 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 (20 – $25^{\circ}C$) for one week. Oocysts were recovered 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). Morphological observations, line drawings, photomicrographs, and measurements were made with the use of an Olympus BX41 binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy) connected to a computer running the software BELView (Version 6.2.3.0, BEL Engineering, Monza, Italy). Line drawings were edited using two software applications from CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), specifically Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

Results

Two *T. flavipes* from the Parque Nacional do Itatiaia, one *T. leucomelas* from Guapimirim and the only *T. amaurochalinus* from Cacaria were positive for an *Isospora* sp. unrecorded in scientific literature.

Isospora brasiliatoae Oliveira and Berto n. sp.

Type host: Yellow-legged thrush *Turdus flavipes* (Vieillot, 1818) (Aves: Passeriformes: Turdidae).

Other hosts: Pale-breasted thrush *Turdus leucomelas* Vieillot, 1818 (Aves: Passeriformes: Turdidae); creamy-bellied thrush *Turdus amaurochalinus* Cabanis, 1850 (Aves: Passeriformes: Turdidae).

Type locality: Parque Nacional do Itatiaia ($22^{\circ}26'57"S$, $44^{\circ}36'25"W$), Southeastern Brazil.

Other localities: Cacaria ($22^{\circ}42'51"S$, $43^{\circ}50'38"W$) and Guapimirim ($22^{\circ}31'10"S$, $43^{\circ}00'36"W$), also from Southeastern Brazil.

Type-material: Photosyntypes, line drawing and oocysts recovered from *T. flavipes* in 2.5% $K_2Cr_2O_7$ 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 MZURPTZ2018007. Photomicrographs are also deposited and available (<http://r1.ufrrj.br/labicoc/colecao.html>) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number P-90/2018. Photographs of the type-host specimen (symbiontotype) are deposited in the same collection.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted

to ZooBank. The Life Science Identifier (LSID) for *Isospora brasilsatoae* is urn:lsid:zoobank.org:act:6C3FEE82-FC9C-4A58-8C17-1713532F03F3.

Site of infection: Unknown.

Prevalence: Four of 10 (40%).

Sporulation: Exogenous. All oocysts were passed in the feces unsporulated and were fully sporulated by day 7 in $K_2Cr_2O_7$ solution at room temperature (20–25°C).

Etymology: The specific name is derived from the family name of a Brazilian parasitologist Dr Marília de Carvalho Brasil Sato, given in her honor for her contribution to the study of taxonomy and ecology of parasites.

Description (Figs 1; 2A-C)

Oocyst ($n = 15$) ovoidal to pyriform, $25\text{--}30 \times 19\text{--}23$ (26.8×21.1); length/width (L/W) ratio 1.2–1.4 (1.27). Wall bi-layered, delicate, 1.4–1.8 (1.6) thick, outer layer smooth, c.2/3 of total thickness. Micropyle present, without micropyle cap, wrinkles or invagination of the inner layer perceptible. Oocyst residuum absent, but 1 to 3 rounded polar granules are present. Sporocyst elongate ovoidal with tapered anterior end (where it is Stieda/sub-Stieda complex), $17\text{--}19 \times 10\text{--}11$ (18.1×10.5); L/W ratio 1.6–1.9 (1.72). Stieda body present, knob-like, 2.0 high \times 2.0 wide. Sub-Stieda body present, trapezoidal, 1.5 high \times 3.5 wide. Para-Stieda body absent. Sporocyst residuum present, as a distinctly sub-spherical body consisting of numerous small granules that appear to be membrane-bound, 6–9 (7.5). Sporozoites vermiform, with anterior and posterior refractile bodies and striations.

Remarks: *Isospora brasilsatoae* is different from other coccidian species of Turdidae of the New World on several aspects (Table 1). It is the only one to have a pyriform shape of the oocysts. It has a micropyle, which is a characteristic present only in the oocysts of *Isospora albicollis* Lainson and Shaw, 1989, *Isospora sabiae* Pinho, Rodrigues, Silva, Lopes, Oliveira, Ferreira, Cardozo, Luz, Ferreira, Lopes and Berto, 2017 and *Isospora machadoae* Pinho, Silva, Rodrigues, Lopes, Oliveira, Luz, Ferreira, Lopes and Berto, 2018 (Lainson & Shaw 1989; Pinho et al. 2017a; 2017b; 2018). From these three, but also from the others, *I. brasilsatoae* can be easily differentiated by having up to three rounded polar granules, sporocysts elongate ovoidal (L/W ratio > 1.6), sub-Stieda body trapezoidal, and compact residuum.

Discussion

Turdus spp. captured in the current study belonged to forest species, which have similar feeding habits, such as eating invertebrates in moist soil and feeding on fruits and berries (Vogel et al. 2014). Thus, the thrushes in the anthropogenic environments may have altered their habits and they had a greater energy expenditure in the adaptation to the different environment, predisposing them to transmissions and infections (Giraudieu et al. 2014). In addition, studies such as de Souza et al. (1992) emphasize that forest environments present smaller birds with predominantly frugivorous feeding habits, which tend to have higher prevalence and densities of coccidia. Despite this, the fecal samples recovered from the thrushes of the current study were not excessively dense, and the birds were apparently healthy, demonstrating that these birds did not suffer from coccidiosis, even in anthropogenic environments.

The coccidian parasites of the host family Turdidae in the New World integrate one of the few groups of coccidia to be well characterized and differentiated in the ‘World of Coccidia’. From the works of McQuistion & Holmes (1988) and Lainson & Shaw (1989) to the most recent works in the 21st century, coccidian species in this host family have been described accurately and in great detail. In this sense, species hardly identified as *I. sabiae*, which required many observations and morphometric evaluations for their characterization (Pinho et al. 2017b), even species with prominent characteristics such as *I. albicollis* and *I. machadoae* are well differentiated (Lainson & Shaw 1989; Pinho et al. 2018), as shown in Table 1.

Isospora brasilsatoae is an example of a coccidian species with very prominent characteristics, which considerably facilitate its identification. In addition to the large size, the pyriform shape that is observed in the great majority of its oocysts, added to the very elongated sporocyst with its tapered end (where it is Stieda/sub-Stieda complex), allow the identification of *I. brasilsatoae* with accuracy.

Finally, the comparison of *I. brasilsatoae* with *Isospora* spp. described from the host family Turdidae in the New World (Table 1) clearly supports the designation as a unique species. Therefore, *I. brasilsatoae* is considered as new to science, being the ninth description in a New World thrush.

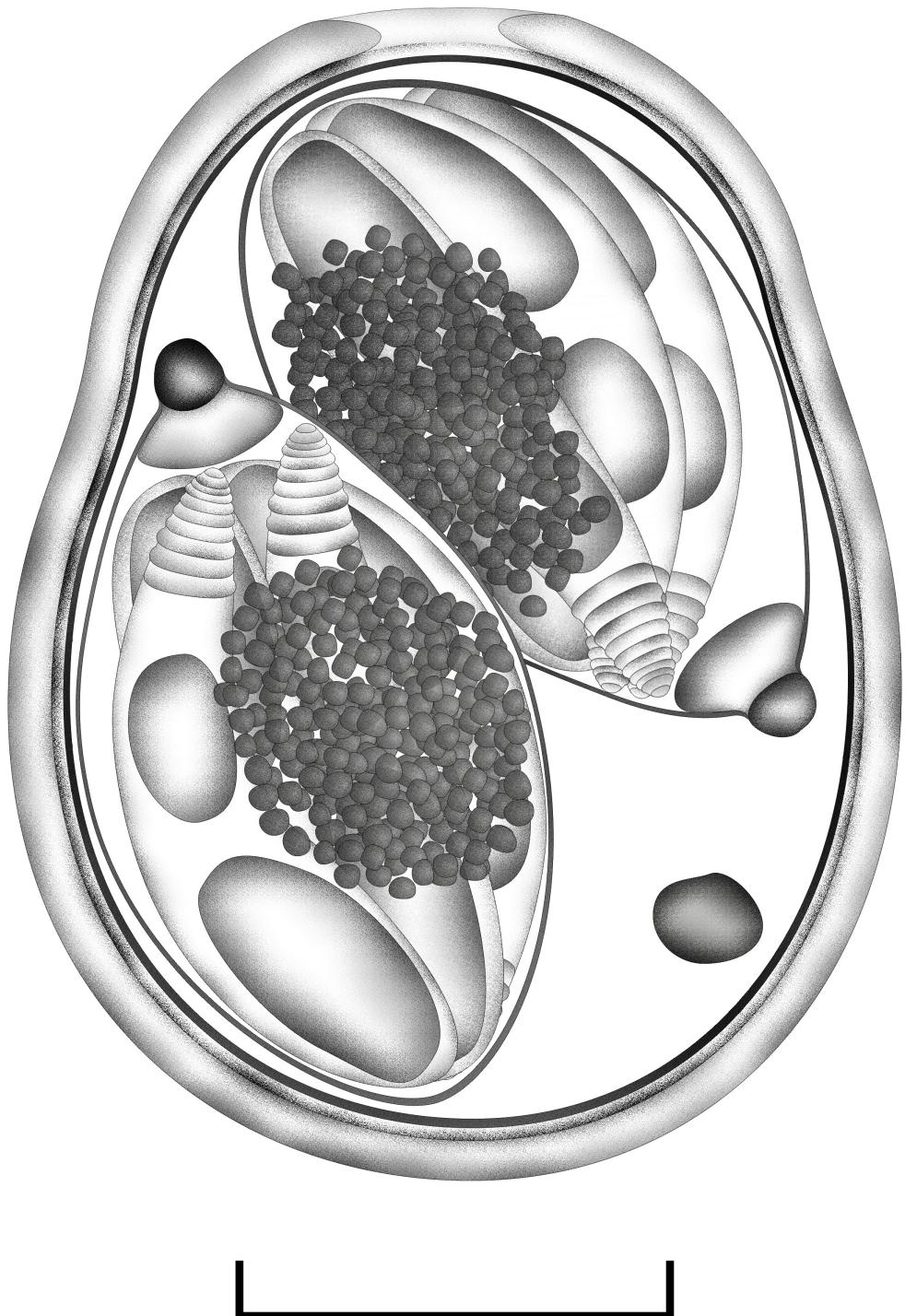


FIGURE 1. Line drawing of the sporulated oocyst of *Isospora brasiliatoae* n. sp, a new coccidium species recovered from the yellow-legged thrush *Turdus flavipes*, the pale-breasted thrush *Turdus leucomelas* and the creamy-bellied thrush *Turdus amaurochalinus*. Scale-bar: 10 μ m.

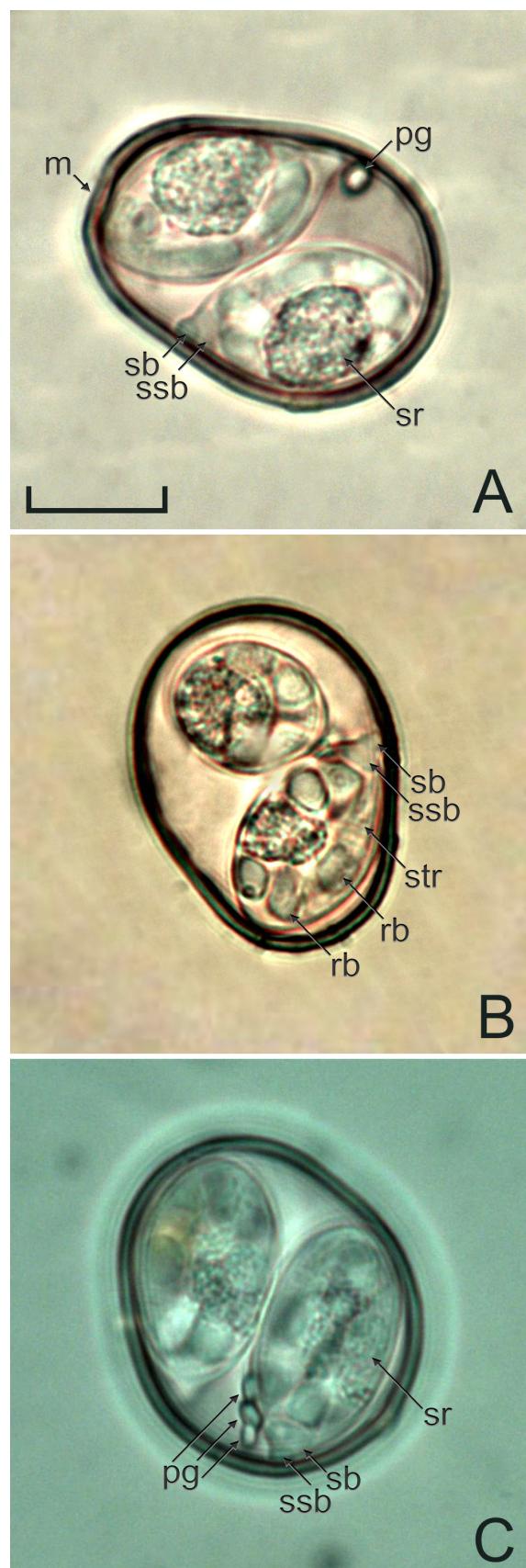


FIGURE 2. Photomicrographs (A-C) of sporulated oocysts of *Isospora brasilsatoae* n. sp., a new coccidium species recovered from the yellow-legged thrush *Turdus flavipes* (A), the pale-breasted thrush *Turdus leucomelas* (B) and the creamy-bellied thrush *Turdus amaurochalinus* (C). Note the micropyle (m), polar granule (pg), Stieda body (sb), sub-Stieda body (ssb), sporocyst residuum (sr), striations (str) and the refractile body (rb). Scale-bar: 10µm.

TABLE 1. Comparative morphology of *Isospora* spp. recorded from Turdidae of the New World

Coccidia	Hosts	References	Oocysts					Sporozoites						
			Shape	Length (μm)	Width (μm)	L/W ratio	Wall	Microyle	Polar granule	Shape	Length (μm)	Width (μm)	L/W ratio	
<i>Isospora phaeornis</i> Levine, Van Riper and Van Riper, 1980	<i>M. modestes obscurus</i> (Gmelin, 1789)	Levine et al. (1980)	ellipsoidal	25–28 (27)	18–20 (19)	—	smooth	absent	present	ovoidal	15–18 (16)	10–11 (11)	—	
<i>Isospora robinii</i> McQuiston and Holmes, 1988	<i>Turdus migratorius</i> Linnaeus, 1766	McQuiston & Holmes (1988)	ellipsoidal or 20–28 ovoid	16–22 (20)	(1.2)	smooth	absent	present	ovoidal	10–17 (13.8)	7–12 (9.0)	(1.5)	nipple-like prominent	
<i>Isospora tucutuensis</i> Lainson and Shaw, 1989	<i>Turdus albicollis</i> Vieillot, 1818	Lainson & Shaw (1989)	sub-spherical	15–19 (17.3)	14–19 (17.1)	—	smooth	absent	present, single, ~3 × 2	ellipsoidal	10–13 (11.8)	7–10 (8.4)	—	nipple-like wide, 0.5 × 1.5 diffuse or compact
<i>Isospora albicollis</i> Lainson and Shaw, 1989	<i>T. albicollis</i>	Lainson & Shaw (1989)	ovoidal	22–27 (24.5)	19–24 (20.3)	—	smooth	present	present, single, ~2.5 × 2	ellipsoidal	12–15 (16.0)	8–10 (11.2)	—	nipple-like to bubble-shaped
<i>I. albicollis</i>	<i>T. albicollis; Turdus leucomelas</i> Vieillot, 1818	Pinho et al. (2017a)	ovoidal	23–26 (24.4)	18–22 (19.7)	1.2–1.4 (1.24)	smooth	present	present, single ellipsoidal	14–17 (15.4)	9–11 (10.1)	1.5–1.6 (1.52)	rounded, 1.7 wide, 1.5 × 3.1	
<i>Isospora zorzali</i> Keefer, Yabsley and Gibbs, McGraw and Hernandez, 2012	<i>Cathartes aurantiirostris</i> Keeler et al. (2012)	round to slightly ovoid	16–24 (19.7)	15–21 (18.6)	(1.1)	smooth	absent	present, 1 to 2	ovoidal	11–18 (14.5)	7–11 (8.5)	(1.7)	nipple-like absent	
<i>Isospora massardi</i> Lopes, Berto, Luz, Galvão, Ferreira and Lopes, 2014	<i>T. albicollis</i>	Lopes et al. (2014)	sub-spherical	15–21 (18.6)	14–19 (17.7)	1.0–1.1 (1.1)	smooth	absent	present, 2 ellipsoidal granules, 1.5 × 0.5	ovoidal	13–16 (14.8)	8–11 (9.3)	1.4–1.8 (1.6)	knob-like rounded, 1 × 2
<i>Isospora sobratai</i> Pinho, Rodrigues, Silva, Lopes, Oliveira, Ferreira, Cardozo, Luz, Ferreira, Lopes and Berto, 2017	<i>T. albicollis; Turdus rufiventris Vieillot, 1818; T. leucomelas; Turdus flavipes Vieillot, 1818</i>	Pinho et al. (2017b)	sub-spherical to ellipsoidal	17–27 (20.9)	15–24 (18.6)	1.0–1.4 (1.12)	present, inconspicuous or imperceptible	—	splinter-like or elongate ellipsoidal reniform	14–22 (16.5)	7–12 (9.2)	1.5–2.1 (1.8)	knob-like, conical, 1.0 × 2.0	
<i>Isospora machadoae</i> Pinho, Silva, Rodrigues, Oliveira, Ferreira, Lopes and Berto, 2018	<i>T. flavipes; T. leucomelas; Turdus amaurochalinus</i> Cabanis, 1850	Pinho et al. (2018)	sub-spherical	21–24 (22.2)	20–23 (21.2)	1.0–1.2 (1.1)	rough	present	present, 1 to 2, sub-spherical and robust	ellipsoidal	12–14 (9.7)	9–11 (9.7)	1.2–1.5 (1.4)	flattened to half-moon-shaped, 0.5 × 1.5
<i>Isospora brasiliatoca</i> Oliveira and Berto n. sp.	<i>T. albicollis</i>	Pinho et al. (2018)	current work	ovoidal to piriform	25–30 (26.8)	19–23 (21.1)	1.2–1.4 (1.27)	smooth	present	elongate ovoidal	17–19 (18.1)	10–11 (10.5)	1.6–1.9 (1.72)	knob-like, trapezoidal, compact

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