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# 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 red-rumped cacique *Cacicus haemorrhous* (L.) (Passeriformes: Icteridae) in southeastern Brazil

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**Abstract** A new species of coccidian, *Isospora guaxi* n. sp., and *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 (Protozoa: Apicomplexa: Eimeriidae) are recorded from red-rumped caciques *Cacicus haemorrhous* (L.) in the Parque Nacional do Itatiaia, Brazil. *Isospora guaxi* n. sp. has sub-spheroidal oöcysts, measuring on average 30.9 × 29.0 μm, with smooth, bi-layered wall *c.*1.9 μm thick. Micropyle and oöcyst residuum are absent, but a polar granule is present. Sporocysts are ellipsoidal, measuring on average 19.3 × 13.8 μm. Stieda body is knob-like and sub-Stieda body is prominent and compartmentalized. Sporocyst residuum is composed of scattered granules. Sporozoites are vermiform, with one refractile body and a nucleus. *Isospora bellicosa* has sub-spheroidal to ovoidal oöcysts, measuring on average 27.1 × 25.0 μm, with smooth, bi-layered wall *c.*1.5 μm thick. Micropyle and oöcyst residuum are absent, but one or two polar granules are present. Sporocysts are ellipsoidal, measuring on average 18.1 × 10.9 μm. Stieda body is knob-like and sub-Stieda body is rounded to rectangular. Sporocyst residuum is composed of a cluster of compact or diffuse granules. Sporozoites are vermiform, with one refractile body and a nucleus. *Isospora bellicosa* was originally described from the Peruvian meadowlark *Sturnella bellicosa* deFilippi, a *trans*-Andean icterid which is allopatric with the *cis*-Andean *C. haemorrhous*. Therefore, in conclusion, this current study reveals the dispersion of coccidia from Icteridae across the Andes Mountains, besides describing the sixth isosporoid coccidium infecting an icterid bird.

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## Introduction

The red-rumped cacique, *Cacicus haemorrhous* (L.) belongs to the family Icteridae. It occurs exclusively in

Neotropical forests of the Amazon region; southeast and midwest regions of Brazil; Paraguay; northeastern Argentina; Colombia; and east of the Andes (Parkes, 1970; BirdLife International, 2015; IUCN, 2015). This species breeds in colonies, weaving nests in the form of suspended bag at the end of tree branches (Feekes, 1981; Sick, 1997).

Species of the family Icteridae are widely distributed, but restricted to the New World, with exception of some species vagrant in Europe, such as the bobolink *Dolichonyx oryzivorus* (L.), the rusty blackbird *Euphagus carolinus* (Müller), the baltimore oriole *Icterus galbula* (L.), the brown-headed cowbird *Molothrus ater* (Boddaert) and the yellow-headed blackbird *Xanthocephalus xanthocephalus* (Bonaparte) (BirdLife International, 2015; IUCN, 2015).

Similar to other families of Passeriformes, the icterids can be parasitised by isosporoid coccidia. Currently, the *Isospora* spp. recorded from passerines of this family are: (i) *Isospora divitis* Pellérdy, 1967 from the Cuban blackbird *Dives atroviolaceus* (d'Orbigny) in Cuba (Pellérdy, 1967); (ii) *Isospora cacici* Lainson, 1994 from the yellow-rumped cacique *Cacicus cela cela* (L.) in Amazonian Brazil (Lainson, 1994); (iii) *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 from the Peruvian meadowlark *Sturnella bellicosa* deFilippi in Peru (Upton et al., 1995); (iv) *Isospora icterus* Upton & Whitaker, 2000 from the Venezuelan troupial *Icterus icterus* (L.) in USA (Upton & Whitaker, 2000); and (v) *Isospora graceannae* Upton & Whitaker, 2000 from the white-edged oriole *Icterus graceannae* Cassin in USA (Upton & Whitaker, 2000).

In this context, this study describes a new species of *Isospora* and *I. bellicosa* from red-rumped caciques *C. haemorrhous* in the Parque Nacional do Itatiaia (PNI) in southeastern Brazil, demonstrating parasite transmission between allopatric icterids that inhabit opposite sides of the Andes.

## Materials and methods

One expedition was conducted in November 2015 in the PNI, which is a protected area with a high degree of vulnerability located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais, and São Paulo. A total of eight *C. haemorrhous* were captured with mist nets at an altitude of 592 m (22°27′48″S, 44°36′10″W). The birds were kept in individual boxes and feces collected immediately after defecation. After identification of the species, the bird was released and stool samples were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v). Samples were carried to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature for one week. Oöcysts 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. (2014a). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope equipped with a digital camera Eurocam 5.0. 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.

### Family Eimeriidae Minchin, 1903
### Genus *Isospora* Schneider, 1881

#### *Isospora guaxi* n. sp.

*Type-host*: Red-rumped cacique *Cacicus haemorrhous* (L.) (Passeriformes: Icteridae).
*Type-locality*: Parque Nacional do Itatiaia (22°27′48″S, 44°36′10″W), southeastern Brazil.
*Type-specimens*: Phototypes and line drawings are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, Seropédica, Rio de Janeiro, Brazil. Photographs of the type-host specimens (symbiotypes) are deposited in the same collection. The repository number is P-64/2016.
*Site in host*: Unknown.
*Prevalence*: 63% (5 out of 8 birds infected).
*Etymology*: The specific epitaph is derived from the common local name for the host, which is 'guaxe'.

Description (Figs. 1A; 2A–C)

*Sporulated oöcyst*
Oöcysts (n = 18) sub-spheroidal, 28–34 × 27–32 (30.9 × 29.0); length/width (L/W) ratio 1.0–1.1

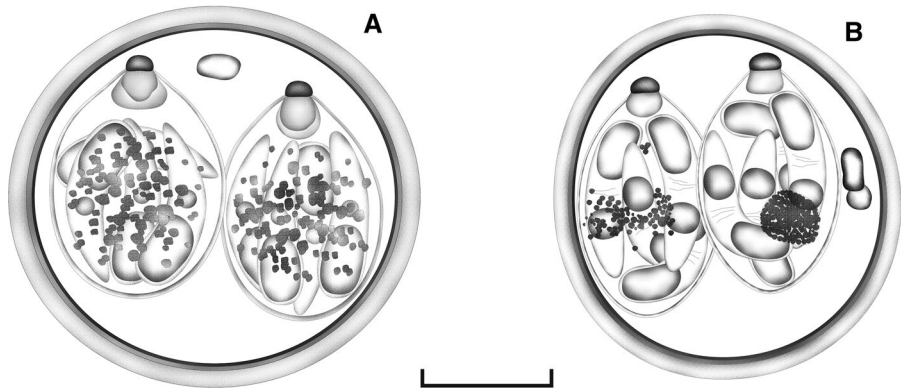


**Fig. 1** Composite line drawings of sporulated oöcysts of *Isospora guaxi* n. sp. (A) and *Isospora bellicosa* (B) ex *Cacicus haemorrhous*. *Scale-bar*: 10 μm

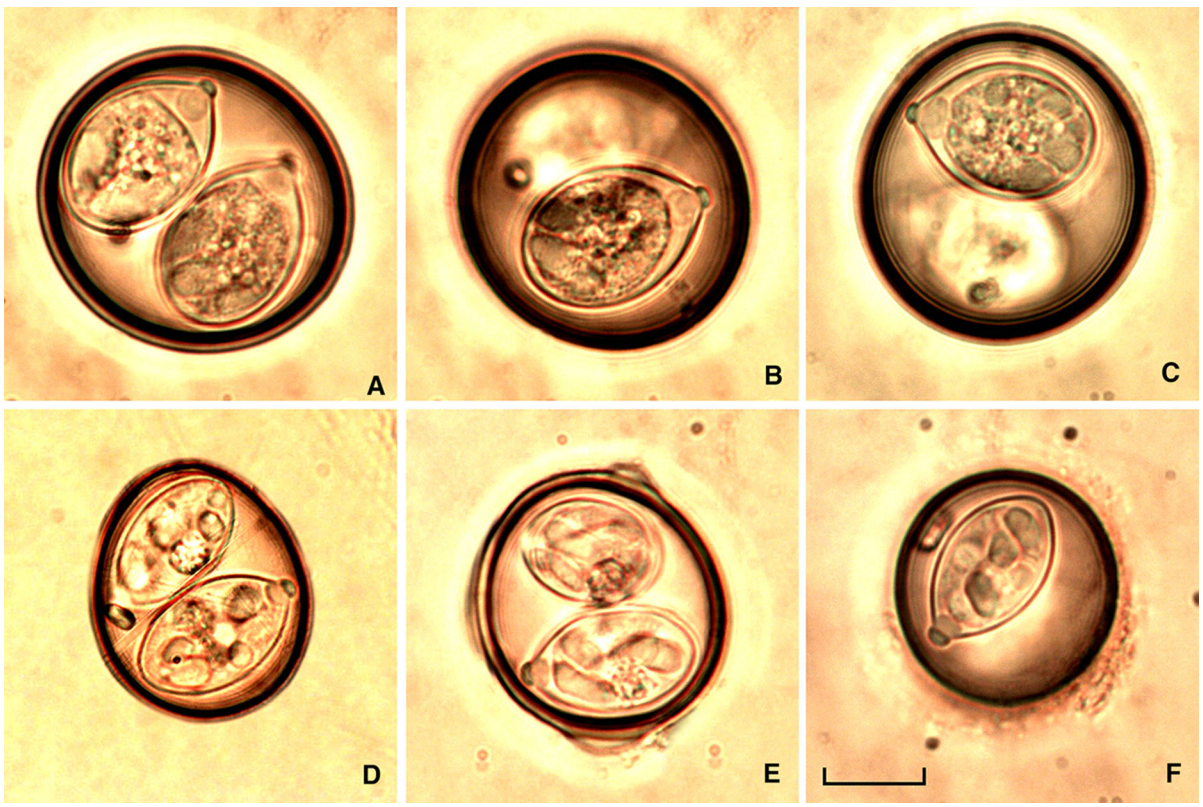


**Fig. 2** Photomicrographs of sporulated oöcysts of *Isospora guaxi* n. sp. (A–C) and *Isospora bellicosa* (D–F) ex *Cacicus haemorrhous*. *Scale-bar*: 10 μm

(1.06). Wall bi-layered, 1.8–2.1 (1.9) thick, outer layer smooth, c.2/3 of total thickness. Micropyle and oöcyst residuum both absent; single polar granule present.

*Sporocyst and sporozoites*

Sporocysts (n = 17) ellipsoidal, 17–21 × 12–15 (19.3 × 13.8); L/W ratio 1.3–1.6 (1.41). Stieda body

present, knob-like, 1.0 high, 2.0 wide; sub-Stieda body present, prominent and compartmentalized with a dense and central portion, rounded or irregular, 2.5 high, 2.0 wide, and a pale portion, rounded or trapezoidal, 3.0 high, 4.0 wide; para-Stieda body absent. Sporocyst residuum present, composed of many scattered granules of different sizes. Sporozoite vermiform with single posterior refractile body and centrally located nucleus.

### *Isospora bellicosa* Upton, Stamper & Whitaker, 1995

*Host*: Red-rumped cacique *Cacicus haemorrhous* (L.) (Passeriformes: Icteridae).
*Locality*: Parque Nacional do Itatiaia (22°27′48″S, 44°36′10″W), southeastern Brazil.
*Material studied*: Phototypes and line drawings are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, Seropédica, Rio de Janeiro, Brazil. Photographs of the type-host specimens (symbiotypes) are deposited in the same collection. The repository number is 65/2016.
*Site in host*: Unknown.
*Prevalence*: 4/8 birds infected (50%).

Description (Figs. 1B; 2D–F)

*Sporulated oöcyst*
Oöcyst (n = 24) sub-spheroidal to ovoidal, 24–32 × 23–27 (27.1 × 25.0); length/width (L/W) ratio 1.0–1.2 (1.08). Wall bi-layered, 1.4–1.7 (1.5) thick, outer layer smooth, *c.*2/3 of total thickness. Micropyle and oöcyst residuum both absent; 1 or 2 polar granules present.

*Sporocyst and sporozoites*
Sporocysts (n = 13), ellipsoidal, 16–21 × 10–12 (18.1 × 10.9); L/W ratio 1.5–1.7 (1.65). Stieda body present, knob-like, 1.5 high, 2.5 wide; sub-Stieda body present, rounded to rectangular, 1.5 high, 2.5 wide; para-Stieda body absent. Sporocyst residuum present, composed of a cluster of compact or diffuse granules. Sporozoite vermiform with one posterior refractile body and centrally located nucleus.

## Discussion

According to Duszynski & Wilber (1997) and an extensive bibliography of coccidia of passerines (Berto et al., 2011), the new species should be compared in detail with the coccidian species which are feature-similar and belong to the same family of the host. Therefore, a comparison was carried out with *Isospora* spp. described from Icteridae (Table 1). The oöcysts of *I. guaxi* n. sp. have similar size only with *I. icterus*; however, the new species can be distinguished by its sub-Stieda body being compartmentalized. This characteristic feature of compartmentalization of the sub-Stieda body is only shared with *I. graceannae*; however, *I. guaxi* has larger oöcysts and sporocysts without splintered granules scattered.

The second morphotype observed in this study was very similar to the morphology described for *I. bellicosa*, except for the shape of oöcysts, which were more ellipsoidal in the description of Upton et al. (1995). However, it would be unwise to consider a new species only based on a slight morphometric difference of the oöcyst, which can be derived from natural polymorphism or a result of environmental and host factors (Fayer, 1980; Gardner & Duszynski, 1990). In any case, it is noteworthy that *I. bellicosa* was originally described from *S. bellicosa* which is allopatric with *C. haemorrhous*. Figure 3A demonstrates the geographic ranges of these icterids *trans*-Andean *S. bellicosa* and *cis*-Andean *C. haemorrhous* with emphasis on the geographical barrier, the Andes Mountains, which separates the populations of these icterids. Similarly, Berto et al. (2014b) report *Isospora sagittulae* McQuistion & Capparella, 1992 from two new *cis*-Andean hosts, *Gymnopithys salvini* (Berlepsch) and *Willisornis poecilinotus* (Cabanis), which are allopatric with the *trans*-Andean host type *Hylophylax naevioides* (Lafresnaye).

The main assumption introduced by Berto et al. (2014b) for dispersion across *trans*- and *cis*-Andean antbirds can be reiterated in the current work. In this thought, icterids with *trans*- and *cis*-Andean distributions could have been infected with *I. bellicosa* in the *trans*-Andean region and transmitted to *C. haemorrhous* in the *cis*-Andean region, dispersing *I. bellicosa* across the Andes. *Cacicus uropygialis* Lafresnaye (Fig. 3B), *C. cela* (Fig. 3C) and *Sturnella militaris* (L.) (Fig. 3D) are congeneric species that have *trans*- and *cis*-Andean distributions sympatric with *S.*

**Table 1** Comparative morphology of *Isospora* spp. recorded from bird hosts of the Icteridae

| Species | Host | Oöcyst | | | | Sporocyst | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Shape | Length × Width | L/W ratio | Polar granule | Length × Width | L/W ratio | Stieda body | Sub-Stieda body | |
| *Isospora guaxi* n. sp. | *Cacicus haemorrhous* (L.) | sub-spheroidal | 28–34 × 27–32 (30.9 × 29.0) | 1.0–1.1 (1.06) | 1 | 17–21 × 12–15 (19.3 × 13.8) | 1.3–1.6 (1.41) | knob-like, 1.0 × 2.0 | prominent and compartmentalized 3.0 × 4.0 | Present study |
| *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 | *Cacicus haemorrhous* (L.) | sub-spheroidal to ovoidal | 24–32 × 23–27 (27.1 × 25.0) | 1.0–1.2 (1.08) | 1–2 | 16–21 × 10–12 (18.1 × 10.9) | 1.5–1.7 (1.65) | knob-like, 1.5 × 2.5 | rounded to rectangular, 1.5 × 2.5 | Present study |
| | *Sturnella bellicosa* deFilippi | ellipsoidal, ovoidal or oblong | 26–32 × 21–26 (29.4 × 23.5) | 1.1–1.4 (1.25) | 1–2 | 17–19 × 10–11 (17.8 × 10.7) | 1.5–1.8 (1.67) | large, 1.5 × 2.5 | homogeneous, 1.5 × 2.5 | Upton et al. (1995) |
| *Isospora divitis* Pellérdy, 1967 | *Dives atroviolaceus* (d'Orbigny) | sub-spheroidal | 22–30 × 20–28 | – | absent | (17 × 13) | – | conspicuous | – | Pellérdy (1967) |
| *Isospora cacici* Lainson, 1994 | *Cacicus cela* (L.) | sub-spheroidal | 22–28 × 20–26 (26.5 × 23.7) | 1.0–1.2 (1.1) | 1–2 | 17–19 × 11–14 (17.7 × 12.5) | 1.2–1.5 (1.4) | prominent, stopper shaped | prominent, 1.0 × 2.0 | Lainson (1994) |
| *Isospora icterus* Upton & Whitaker, 2000 | *Icterus icterus* (L.) | sub-spheroidal | 27–32 × 25–30 (28.9 × 27.2) | 1.0–1.1 (1.06) | 1 | 17–19 × 12–14 (17.8 × 12.8) | 1.3–1.5 (1.39) | nipple-like, 1.5 × 2.0 | homogeneous, flask-shaped, 3.0 × 5.0 | Upton & Whitaker (2000) |
| *Isospora graceannae* Upton & Whitaker, 2000 | *Icterus graceannae* Cassin | sub-spheroidal | 20–26 × 19–25 (23.9 × 22.3) | 1.0–1.2 (1.07) | 1, splinter with age | 14–16 × 10–11 (15.5 × 10.7) | 1.4–1.5 (1.44) | stout, 1.0 × 1.0 | compartmentalized 2.5 × 3.0 | Upton & Whitaker (2000) |

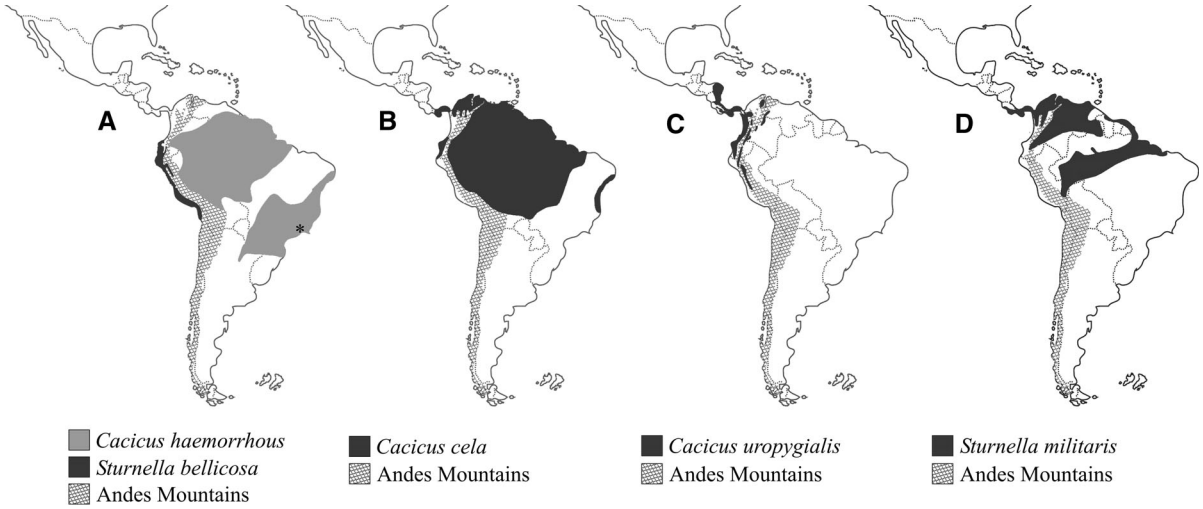


**Fig. 3** Geographic range of several *Cacicus* spp. and *Sturnella* spp. in South America [adapted from De las Casas et al. (2004), BirdLife International (2015) and IUCN (2015)]. Hosts of *Isospora bellicosa* are Peruvian meadowlarks *Sturnella bellicosa* and red-rumped caciques *Cacicus haemorrhous*. *Sturnella bellicosa* is allopatric with *C. haemorrhous*, besides being separated by the Andes (A). Yellow-rumped cacique *Cacicus cela* (B), scarlet-rumped cacique *Cacicus uropygialis* (C) and red-breasted blackbird *Sturnella militaris* (D) are examples of *trans-* and *cis*-Andean icterid birds that are sympatric with *S. bellicosa* and (or) *C. haemorrhous*. The asterisk indicates the approximate location of the collection point of the current study

*bellicosa* and *C. haemorrhous*; therefore, these species are examples of possible dispersers of *I. bellicosa* across the Andes. Additionally, it is noteworthy that some ornithological studies have demonstrated the ability of several passerines to cross or bypass the Andes Mountains especially in recent decades, as a consequence of the accelerated rate of habitat loss and fragmentation along the Andean slopes and adjacent lowlands, coupled with the effect of global warming on the geographical ranges of the species (De las Casas et al., 2004; Avendaño et al., 2013).

It is also important to note that the red-rumped caciques infected in the current work were captured in southeastern Brazil (Fig. 3A), at the opposite end side of South America. This south-eastern population of *C. haemorrhous* is allopatric with the north-western population, which is close to the populations of *Sturnella* spp. In this sense, it is concluded that *Cacicus* spp., such as *C. cela*, or other icterids susceptible to *I. bellicosa* that have geographical ranges in central Brazil should have transmitted *I. bellicosa* to the south-eastern population of *C. haemorrhous*.

In conclusion, *I. guaxi* is considered as new to science and the sixth isosporoid coccidium reported from an icterid bird. Additionally, a new host for *I. bellicosa* is recorded once sporulated oöcysts with similar features were recovered from *C. haemorrhous* supporting parasite transmission between allopatric birds of same family and different genera that inhabit opposite sides of the Andes.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Field collecting permits were issued to B.P. Berto by SISBIO/ICMBio (license No. 49605–1) and CEUA/ICBS/UFRRJ (protocol No. 008/2015).

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