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# *Isospora celata* n. sp. (Apicomplexa: Eimeriidae) from the orange-crowned warbler *Oreothlypis celata* (Say) (Passeriformes: Parulidae) in Mexico

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**Abstract** A new coccidian species (Protista: Apicomplexa: Eimeriidae) is described from the orange-crowned warbler *Oreothlypis celata* (Say) collected in the Nevado de Toluca National Park, Mexico at 3,000 metres above sea level. *Isospora celata* n. sp. has subspheroidal oöcysts, measuring 28.4 × 26.4 μm, with smooth, bi-layered wall *c*.1.2 μm thick. Micropyle and polar granule are absent, but oöcyst residuum is present as a compact mass. Sporocysts are ovoidal, 18.2 × 12.8 μm. Stieda body knob-like and sub-Stieda body irregular and barely discernible. Sporocyst residuum is composed of granules of different sizes. Sporozoites are vermiform with one refractile body and a nucleus. This is the third description of an isosporoid coccidian infecting a New World warbler.

## Introduction

The warblers (Family Parulidae) are passerines of the New World, occupying the same ecological niche as silvid passerines. They are small and often very colourful passerines. The detail and colour of plumage vary between species. The warblers are mainly insectivores, supplementing their diet with berries and fruits (Lovette & Bermingham, 2002; IUCN, 2014).

The orange-crowned warbler *Oreothlypis celata* (Say), is a Neotropical migrant that breeds in a variety of open woodland habitat types, ranging from edges of evergreen forests in Alaska to oak scrublands in California. In winter, this species utilizes similar kinds of open habitats up to Central America. In Mexico, *O. celata* overwinters at the Nevado de Toluca National Park coniferous forest, a protected natural area of the

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State of Mexico, Mexico (Sánchez-Jasso et al., 2013; IUCN, 2014).

To date, only two coccidia were described from warblers. Berto et al. (2009) described *Isospora piacobrai* Berto, Flausino, Luz, Ferreira & Lopes, 2009 from the masked yellowthroat *Geothlypis aequinoctialis* (Gmelin) in Brazil and Keeler et al. (2014) described *Isospora orbisreinitas* Keeler, Yabsley, Adams & Hernandez, 2014 from the rufous-capped warbler *Basileuterus rufifrons* (Swainson) and from the ovenbird *Seiurus aurocapilla* (Linnaeus) in Costa Rica. This paper describes the third coccidian species infecting the orange-crowned warbler *O. celata* in Mexico.

## Materials and methods

A total of four orange-crowned warblers were captured on November 1st (2 specimens) and December 18th (2 specimens) 2013, by using seven mist nets from 6:00 to 3:00 pm in the Parque Ecológico Ejidal de Cacalomacán located into the Nevado de Toluca National Park (19°12′37″N, 99°44′42″W; 19°12′31″N, 99°43′51″W; 19°11′31″N, 99°44′22″W; 19°11′47″N, 99°′09″W), State of Mexico, Mexico (Sánchez-Jasso et al., 2013). The passerines were kept for 5–10 minutes in individual bags and faeces were collected immediately after defecation. After the species identification, morphometric data were obtained, determining plumage patters and banding them with USGS Bird Banding Laboratory (BBL) bands as part of the MoSI programme of the Institute of Bird Population (DeSante et al., 2005). Then, the birds were released and the faecal samples were placed in plastic vials containing 2.5% potassium dichromate solution ($K_2Cr_2O_7$) 1:6 (v/v). Samples were sent to the Laboratório de Coccídios e Coccidioses, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were placed in a thin layer (*c*.5 mm) of $K_2Cr_2O_7$ 2.5% solution in Petri dishes, incubated at 23–28°C and monitored daily, until 70% of oöcysts were sporulated. Oöcysts were recovered by flotation in Sheather's sugar solution (S.G. 1.20) and microscopically examined using the technique described by Duszynski & Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope coupled to a digital camera Eurocam 5.0. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

## Results

Four orange-crowned warblers (*O. celata*) were examined; two of them shed oöcysts in the faeces (bands 2530707578 and 253070578). Initially, the oöcysts were non-sporulated, but approximately 70% of the oöcysts were sporulated at day 2 (under the conditions used in this study).

### *Isospora celata* n. sp.

*Type-host*: *Oreothlypis celata* (Say) (Aves: Passeriformes: Parulidae).
*Type-specimens*: Phototypes and line drawings of sporulated oöcysts are deposited and available in the Parasitology Collection of the Laboratório de Coccídios e Coccidioses, at the UFRRJ, Seropédica, Rio de Janeiro, Brazil (see also http://r1.ufrrj.br/lcc). Photographs of the type-host specimens (symbiotypes) are deposited in the same collection (repository number P-55/2014).
*Type-locality*: Nevado de Toluca National Park (19°12′37″N, 99°44′42″W; 19°12′31″N, 99°43′51″W; 19°11′31″N, 99°44′22″W; 19°11′47″N, 99°45′09″W), State of Mexico, Mexico.
*Sporulation time*: Two days.
*Site of infection*: Not investigated.
*Etymology*: The specific epithet is derived from the specific name of the type-host.

Description (Fig. 1A–D)

*Sporulated oöcyst*
Oöcyst (n = 20) subspheroidal, 27–30 × 25–28 (28 × 26); length/width (L/W) ratio 1.0–1.1 (1.1). Wall bilayered, 1.0–1.3 (1.2) thick, outer layer smooth, *c*.2/3 of total thickness. Micropyle and polar granule both absent, oöcyst residuum present as a compact mass.
*Sporocyst and sporozoites*
Sporocysts (n = 20) 2, ovoidal, 15–20 × 11–14 (18 × 13); L/W ratio 1.4–1.5 (1.4). Stieda body present, knob-like, 1.0 high, 2.5 wide; sub-Stieda body present, irregular, barely discernible, 1.5 high, 4.0 wide; para-Stieda body absent; sporocyst residuum present,

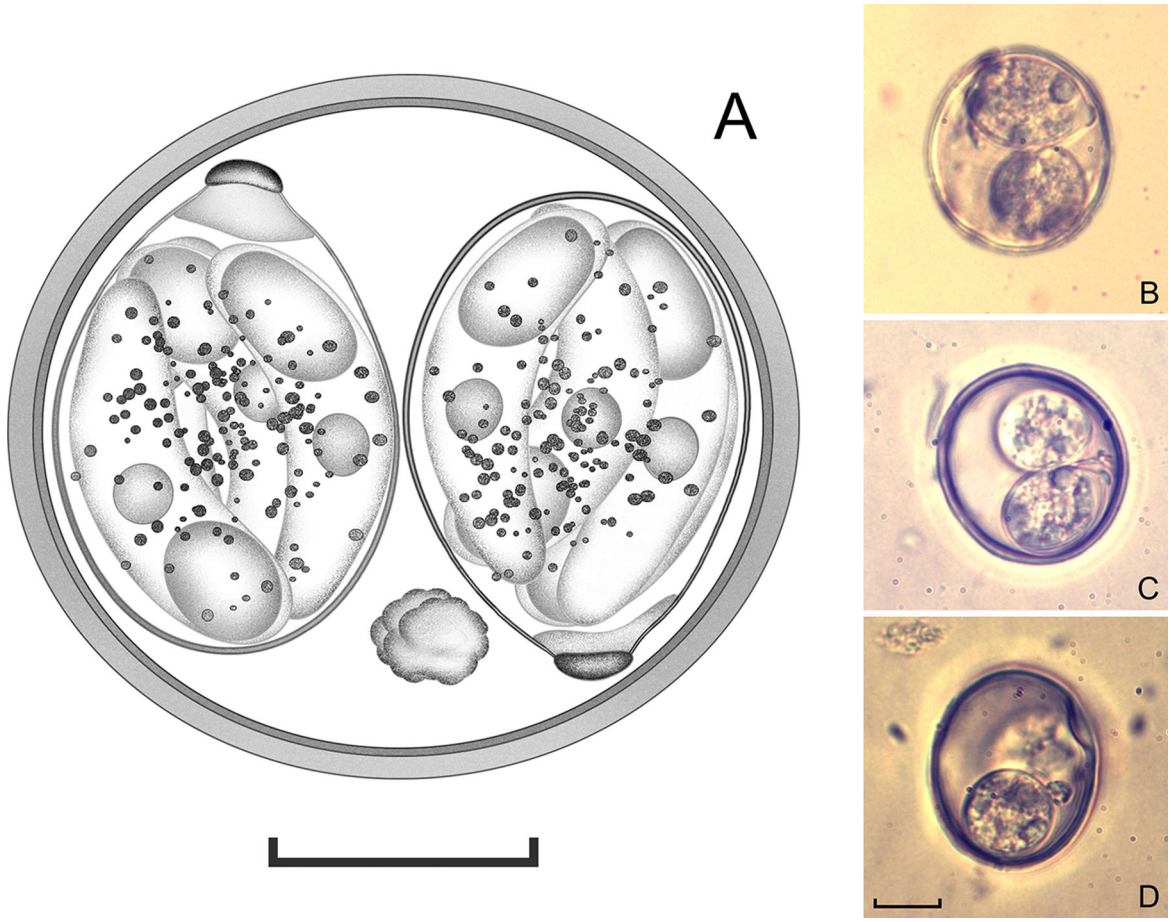


**Fig. 1** Oöcysts of *Isospora celata* from the orange-crowned warbler *Oreothlypis celata*. A, Composite line drawing; B–D, Photomicrographs. *Scale-bars*: 10 μm

consisting of scattered spherules of different sizes. Sporozoites 4, vermiform, with single posterior refractile body and centrally located nucleus.

## Discussion

One hundred and fifteen warblers occur in the New World, of which only five were reported as hosts of *Isospora* (IUCN, 2014). In addition to the two hosts of *I. orbisreinitas* (see Keeler et al., 2014) and one host for *I. piacobrai* (see Berto et al., 2009), undescribed isosporoid coccidia were reported from the common yellowthroat *Geothlypis trichas* (Linnaeus) (Boughton et al., 1938) and the Nashville warbler *Oreothlypis ruficapilla* (Wilson) (Swayne et al., 1991). This low frequency may not reflect the distribution and prevalence of *Isospora* spp. in New World warblers, but because of the few studies on the coccidia from Parulidae, only these few species have been reported and/or described (Berto & Lopes, 2013).

The coccidium of this current study was compared in detail with coccidian parasites of New World passerine birds that are morphologically similar and belong to the same host family (Duszynski & Wilber 1997; Berto et al., 2011). Based on Table 1, it can be concluded that *I. celata* is differentiated using the morphology and morphometry of the oöcysts from the species of *Isospora* in the New World passerines of same family. Therefore, *I. celata* is considered as a species new to science, being the third species of the genus described from the Parulidae.

The orange-crowned warbler is a Neotropical migrant usually found in mixed migration and foraging

**Table 1** Comparative morphology of *Isospora* spp. recorded from warblers (Parulidae)

| Species | *Isospora celata* n. sp. | *Isospora piacobrai* Berto, Flausino, Luz, Ferreira & Lopes, 2009 | *Isospora orbisreinitas* Keeler, Yabsley, Adams & Hernandez, 2014 |
|---|---|---|---|
| Host | *Oreothlypis celata* (Say) | *Geothlypis aequinoctialis* (Gmelin) | *Basileuterus rufifrons* (Swainson) |
| Reference | Present study | Berto et al. (2009) | Keeler et al. (2014) |
| *Oöcyst* | | | |
| Shape | subspheroidal | subspheroidal to ovoidal | spheroidal to ovoidal |
| Length | 27–30 (28.4) | 21–26 (23.5) | 21–28 (24.3) |
| Width | 25–28 (26.4) | 20–24 (21.6) | 19–25 (22.3) |
| Length/Width ratio | 1.0–1.1 (1.1) | 1.1–1.1 (1.1) | 1.0–1.3 (1.0) |
| Polar granule | absent | present, 1 | present, 0–4, spherical to cigar-shaped |
| Oöcyst residuum | present, compact mass | absent | absent |
| *Sporocyst* | | | |
| Shape | ovoidal | ovoidal | ovoidal |
| Length | 15–20 (18.2) | 15–17 (15.8) | 12–19 (16.0) |
| Width | 11–14 (12.8) | 9–12 (10.5) | 10–14 (11.8) |
| Length/Width ratio | 1.4–1.5 (1.4) | 1.4–1.6 (1.5) | 1.0–1.9 (1.4) |
| Stieda body | knob-like | prominent; knob-like | knob-like |
| Sub-Stieda body | irregular; barely discernible | large; trapezoidal; homogeneous | prominent; trapezoidal; compartmentalised |
| Residuum | diffuse | diffuse | diffuse |

flocks (Hutto, 1987; Gram, 1998). Despite the specificity of some isosporoid parasites, a chance of cross-species infection with other Neotropical migrants in the wintering grounds exist (Keeler et al., 2014), but also in opposite direction, considering the chance of infection from wintering to breeding grounds.

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