


Isospora cardellinae n. sp. (Apicomplexa: Eimeriidae) from the red warbler *Cardellina rubra* (Swainson) (Passeriformes: Parulidae) in Mexico

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Abstract A new coccidian species (Protozoa: Apicomplexa: Eimeriidae) collected from the red warbler *Cardellina rubra* (Swainson) is reported from the Nevado de Toluca National Park, Mexico. *Isospora cardellinae* n. sp. has subspherical oöcysts, measuring on average $26.6 \times 25.4 \mu\text{m}$, with smooth, bi-layered wall, c. $1.3 \mu\text{m}$ thick. Micropyle, oöcyst residuum, and polar granule are absent. Sporocysts are ovoidal, measuring on average $19.0 \times 12.0 \mu\text{m}$, with a knob-like Stieda body, a trapezoidal sub-Stieda body and sporocyst residuum composed of scattered spherules of different sizes. Sporozoites are vermiform with one refractile body and a nucleus. This is the fourth

description of an isosporoid coccidian infecting a New World warbler.

Introduction

The New World warblers are passerines in the family Parulidae (Lovette & Bermingham, 2002). The warblers are small, often very colourful, mainly insectivorous passerine birds with a broad diversity of habitat affinities and life histories (IUCN, 2015). The red warbler *Cardellina rubra* (Swainson) is nearly all red with a silvery-white cheek patch, endemic to highland mountains of Mexico (Swainson, 1827; Peterson & Chalif, 1973; Barrera-Guzmán et al., 2012).

To date, only three coccidia have been described from warblers: *Isospora piacobrai* Berto, Flausino, Luz, Ferreira & Lopes, 2010 described from the masked yellowthroat *Geothlypis aequinoctialis* (Gmelin) in Brazil (Berto et al., 2010); *I. orbisreinitas* Keeler, Yabsley, Adams & Hernandez, 2014 described from the rufous-capped warbler *Basileuterus rufifrons* (Swainson) and from the ovenbird *Seiurus aurocapilla* (Linnaeus) in Costa Rica (Keeler et al., 2014); and *Isospora celata* Berto, Medina, Salgado-Miranda, García-Conejo, Janczur, Lopes & Soriano-Vargas, 2014 described from the orange-crowned warbler *Oreothlypis celata* (Berto et al., 2014b).

Only unsporulated coccidian oöcysts have been observed in red warblers *C. rubra* from the Nevado de

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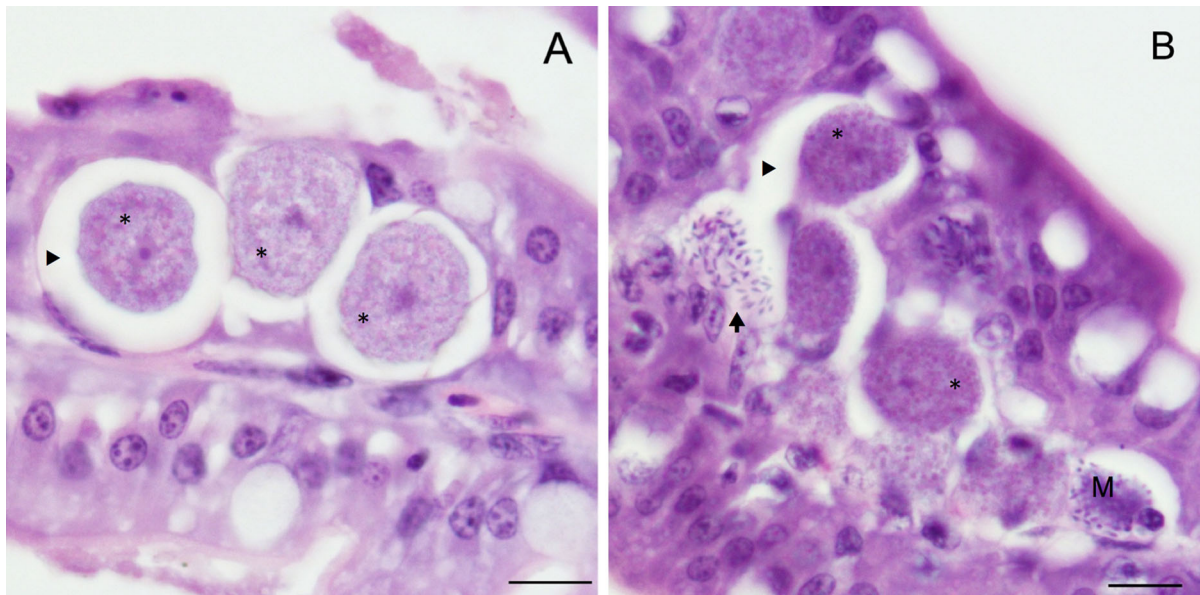


Fig. 1 *Isospora cardellinae* from the intestine of the red warbler *Cardellina rubra* in Mexico. A, Histological section of the duodenum, showing macrogamonts with centrally located nuclei (*), surrounded by a parasitophorous vacuole (arrowhead); B, Histological section of the jejunum showing a partial section of a meront with merozoites (arrow), macrogamonts (*) and microgamonts (M). Scale-bars: 10 μ m

Toluca National Park coniferous forest, a protected natural area of the State of Mexico, Mexico (Medina et al., 2015). This paper describes the fourth coccidian species infecting the New World red warbler *C. rubra*, and the second species of *Isospora* Schneider, 1881 identified in passerines from the Nevado de Toluca National Park, State of Mexico, Mexico.

Materials and methods

Red warblers were captured during 17, three-day-samplings, from January 7th, 2014 to July 10th, 2015, with the use of seven mist nets, from 6:00 a.m. through 3:00 p.m., in the Parque Ecológico Ejidal de Cacalocacán located in 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°45'09"W), State of Mexico, Mexico (Sánchez-Jasso et al., 2013). The passerines were placed for for 5–10 minutes into individual bags and faeces were collected immediately after defecation. Birds were banded, morphometric data obtained and moulting patterns determined as part of the MoSI programme of the Institute of Bird

Population (DeSante et al., 2005). 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). In the laboratory, the 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. (2014a). Morphological observations, line drawings, photomicrographs and measurements were made using a Nikon Eclipse 80i binocular microscope equipped with a digital camera Nikon DS-Fi2. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

To investigate the intestinal site of infection, one infected bird was euthanatized. Visceral samples were fixed in 10% (v/v) neutral buffered formalin (pH 7.4) and embedded in paraffin. The intestine was cut in ring sections all along its length. Serial sections (c.5 μ m thick) were stained with haematoxylin and eosin.

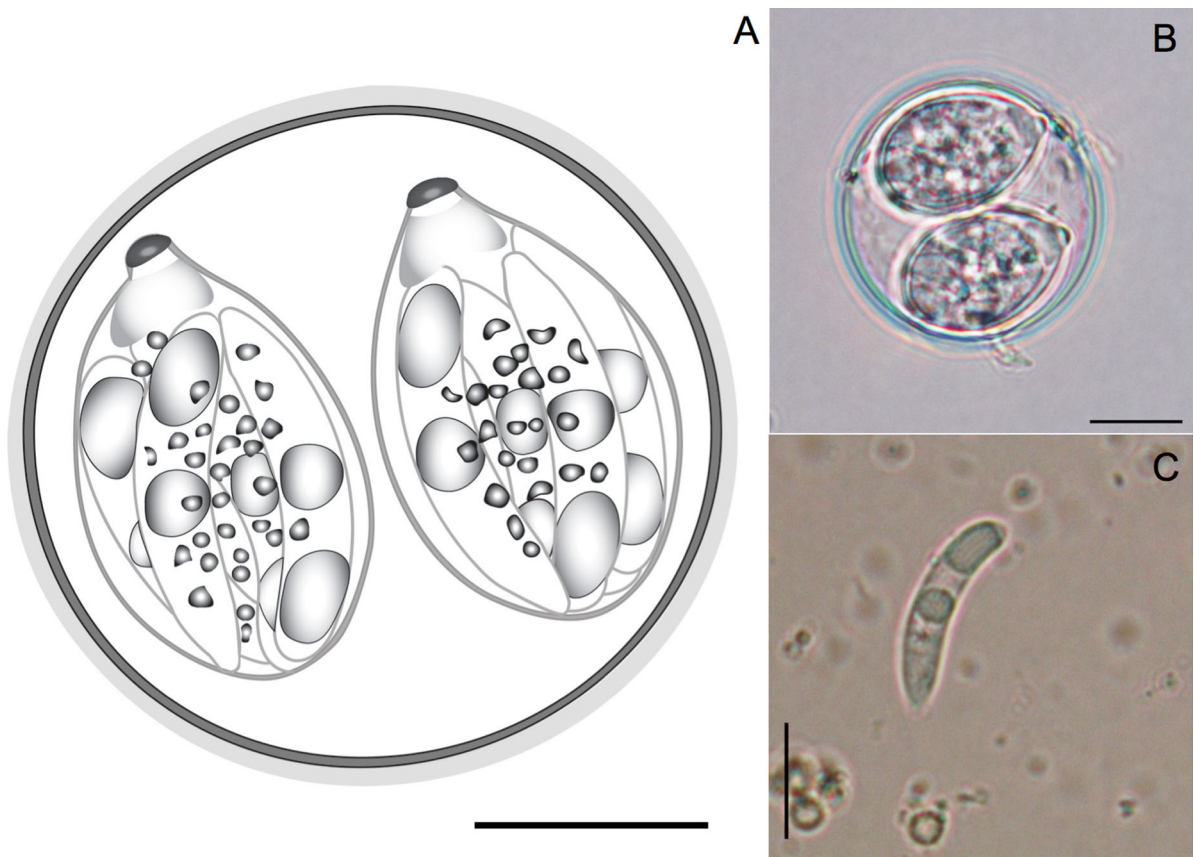


Fig. 2 Oocysts of *Isospora cardellinae* from the red warbler *Cardellina rubra*. A, Composite line drawing; B, C, Photomicrographs. Scale-bars: 10 μ m

Results

A total of 41 red warblers (*C. rubra*) were captured; of these 34 were examined. Six of the 34 shed oocysts in the faeces. Initially, the oocysts were non-sporulated, but approximately 70% of the oocysts were sporulated at day 2 (under the conditions used in this study).

Isospora cardellinae n. sp.

Type-locality: Nevado de Toluca National Park (19°12'09"N, 99°44'51"W), State of Mexico, Mexico.

Other localities: 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°45'09"W), State of Mexico, Mexico.

Type-material: Prototypes and line drawings of sporulated oocysts and histological slides containing

endogenous forms are deposited in the Parasitology and Bacteriology Collection of the Laboratory of Avian Microbiology, Centro de Investigación y Estudios Avanzados en Salud Animal. Photographs of the type-host specimens (syntypes) are deposited in the same collection. The repository number is ESV-22/2015.

Sporulation time: Two days.

Site in host: Epithelial cells along the length of the villi of duodenum and jejunum (Fig. 1A–B).

Etymology: The specific epithet is derived from the genus name of the type-host.

Description (Figs. 1, 2)

Sporulated oocyst

Oocyst ($n = 23$) subspherical, 23–28 \times 23–27 (26.6 \times 25.4); length/width (L/W) ratio 1.0–1.1 (1.1). Wall bilayered, 1.2–1.4 (1.3) thick, outer layer smooth, $c.$ 1/3

Table 1 Comparative morphology of *Isoxpora* spp. recorded from warblers (Parulidae) from the Americas

Species	<i>I. cardellinae</i> n. sp.	<i>I. celata</i> Berto, Medina, Salgado-Miranda, Garcia-Conejo, Janczur, Lopes & Soriano-Vargas, 2014	<i>I. piacobrai</i> Berto, Flausino, Luz, Ferreira & Lopes, 2009	<i>I. orbisreinitas</i> Keeler, Yabsley, Adams & Hernandez, 2014
Host	<i>Cardellina rubra</i> (Swainson) Present study	<i>Oreothlypis celata</i> (Say) Berto et al. (2014)	<i>Geothlypis aequinoctialis</i> (Gmelin) Berto et al. (2009)	<i>Basileuterus rufifrons</i> (Swainson) Keeler et al. (2014)
Reference				
<i>Oöcyst</i>				
Shape	subspherical	subspherical	subspherical to ovoidal	spherical to ovoidal
Length	23–28 (26.6)	27–30 (28.4)	21–26 (23.5)	21–28 (24.3)
Width	23–27 (25.4)	25–28 (26.4)	20–24 (21.6)	19–25 (22.3)
Length/Width ratio	1.0–1.1 (1.1)	1.0–1.1 (1.1)	1.1–1.1 (1.1)	1.0–1.3 (1.0)
Polar granule	absent	absent	present, 1	present, 0–4, spherical to cigar-shaped
Oöcyst residuum	absent	present, compact mass	absent	absent
<i>Sporocyst</i>				
Shape	ovoidal	ovoidal	ovoidal	ovoidal
Length	18–20 (19.0)	15–20 (18.2)	15–17 (15.8)	12–19 (16.0)
Width	11–13 (12.0)	11–14 (12.8)	9–12 (10.5)	10–14 (11.8)
Length/Width ratio	1.6–1.8 (1.7)	1.4–1.5 (1.4)	1.4–1.6 (1.5)	1.0–1.9 (1.4)
Stieda body	knob-like	knob-like	prominent; knob-like	knob-like
Sub-Stieda body	trapezoidal, irregular base	irregular; barely discernible	large; trapezoidal; homogeneous	prominent; trapezoidal; compartmentalized
Residuum	diffuse	diffuse	diffuse	diffuse

of total thickness. Micropyle, polar granule, and oöcyst residuum are all absent.

Sporocyst and sporozoites

Sporocysts (n = 23) 2, ovoidal, 18–20 × 11–13 (19.0 × 12.0); L/W ratio 1.6–1.8 (1.7). Stieda body present, knob-like, 1.1 high × 2.4 wide; sub-Stieda present, trapezoidal, rounded, and sometimes with irregular base, 1.8 high × 4.5 wide; para-Stieda body absent; sporocyst residuum present, consisting of scattered spherules of different sizes. Sporozoites 4, vermiform, with single posterior refractile body and centrally located nucleus (Fig. 2).

Endogenous forms

Histopathological examination of tissues helped detect endogenous stages in the epithelial cells of the duodenum and jejunum (Fig. 1). Endogenous stages develop extranuclearly in the cytoplasm of epithelial cells. Most of the endogenous stages were observed mainly into epithelial cells along the length of the villi of duodenum. Gamogonic stages were differentiated into microgamonts and macrogamonts. Microgamonts were ovoidal and measured 14 × 10 µm, producing curved merozoites measuring 1.9 × 0.5 µm. The subspherical macrogamonts measured 25 × 23 µm and were characterized by a centrally located nuclei.

Discussion

Of the 115 warbler species that occur in the New World, only five have been reported as hosts of *Isoospora* spp.: *Oreothlypis celata* (Say) for *I. celata* (see Berto et al., 2014); *Basileuterus rufifrons* (Swainson) for *I. orbisreinitas* (see Keeler et al., 2014); *Geothlypis aequinoctialis* (Gmelin) for *I. piacobrai* (see Berto et al., 2010), and the common yellowthroat *Geothlypis trichas* (Linnaeus) (Boughton et al., 1938) and the Nashville warbler *Oreothlypis ruficapilla* (Wilson) for an undescribed isosporoid coccidian (Swayne et al., 1991). This low frequency may not reflect the distribution and prevalence of *Isoospora* spp. in the New World warblers, but is rather an outcome of a small number of studies on the genus *Isoospora* from Parulidae (Berto & Lopes, 2013).

The sporulated oocysts obtained in this study were compared in detail with coccidian parasites from other New World passerine birds that are feature-similar and belong to the same host family (Duszynski & Wilber

1997; Berto et al., 2014b; see Table 1). The morphology and morphometry of the oocysts of *I. cardellinae* allow differentiating it from other *Isoospora* species. An oöcyst residuum is present in *I. celata* (Berto et al., 2014), and a polar granule is present in both *I. piacobrai* and *I. orbisreinitas* (Berto et al., 2010; Keeler et al., 2014). In conclusion, we studied and described here a fourth evidence of *Isoospora* genus in a New World warbler species. The histopathological study demonstrated the *Isoospora* intestinal infection, in which various life-cycle stages were detected, in the red warbler *C. rubra*.

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Compliance with ethical standards

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

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed. Collecting wildlife permit in Mexico provided by SEMARNAT (code SGPA/DGVS/07613/14).

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