



Isospora speciosae n. sp. (Apicomplexa: Eimeriidae) from the black-pollled yellowthroat *Geothlypis speciosa* Sclater, 1859 (Passeriformes: Parulidae) in Ciénegas del Lerma, Mexico

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Received: 16 March 2023 / Accepted: 30 May 2023 / Published online: 11 June 2023
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Abstract A new coccidian species, *Isospora speciosae* n. sp. (Apicomplexa: Eimeriidae) collected from the black-pollled yellowthroat *Geothlypis speciosa* Sclater, is reported from the marsh Ciénegas del Lerma Natural Protected Area, Mexico. Sporulated oöcysts of the new species are subspherical to ovoidal, 24–26 × 21–23 (25.7 × 22.2) µm, with a length/width (L/W) ratio of 1.1; one or two polar granules are present, but micropyle and oöcyst residuum are absent. Sporocysts are ovoidal, 17–19 × 9–11 (18.7 × 10.2) µm, with a L/W ratio of 1.8; Stieda and sub-Stieda bodies are both present, but para-Stieda body is absent; sporocyst residuum compact. The new species is the sixth species of *Isospora* recorded in a bird of the family Parulidae in the New World.

Introduction

The endemic, black-pollled yellowthroat (*Geothlypis speciosa*) is a New World warbler that resides in freshwater marshes, known just from four areas in

central Mexico, in the states of Guanajuato, Mexico and Michoacan (Pérez-Arteaga et al., 2018). The species is listed as globally vulnerable by BirdLife International (2023), and under the Mexican legislation, at risk of extinction (DOF, 2010). The male of this parulid has a broad black mask on the face, a black forecrown, but the mask does not have a pale upper border as in other yellowthroats. The female is olive, brown on the dorsum and yellow on the venter, with dusky olive flanks (Antolin & Ghalambor, 2020). Two subspecies, *G. s. limnatis* and *G. s. speciosa*, are recognized (Dickerman, 1970).

Up to now, five species of *Isospora* has been identified in parulids of the New World: *I. cardellinae* in the red warbler (*Cardellina rubra*) (Salgado-Miranda et al., 2016) and *I. celata* in the orange-crowned warbler (*Leiothlypis celata*) (Berto et al., 2014b) in Mexico, *I. piacobrai* in the masked yellowthroat (*Geothlypis aequinoctialis*) (Berto et al., 2010) and *Isospora basileuterusi* in the *Basileuterus culicivorus* (Mello et al., 2022) in Brazil and *I. orbisreinitas* in the rufous-capped warbler (*Basileuterus rufifrons*) in Costa Rica (Keeler et al., 2014). An undescribed isosporoid coccidia has been reported in the common yellowthroat *Geothlypis trichas* (Boughton et al., 1938) and in the Nashville warbler *Leiothlypis ruficapilla* (Swayne et al., 1991). In Mexico, *C. rubra*, *L. celata*, *B. rufifrons* and *G. trichas*, overlap in their distribution with *G. speciosa* subsp. *speciosa*. Only *L. celata* and *G. trichas* have

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been recorded in the marshes Ciénegas del Lerma (Soriano-Vargas, 2016).

Materials and methods

During daily trips to marshes Ciénegas del Lerma (19°20′24″N, 99°29′26″W; 19°21′28″N, 99°31′10″W), Mexico, black-pollled yellowthroats (*G. speciosa*) were observed and photographed, from April 15, 2020 to May 15, 2021. Birds were observed eating, moving and perching atop California bulrush (*Schoenoplectus californicus*) and reed (*Thypha* sp.). During photographic sessions at different locations, 5

adult males and 2 females left droppings on the leaves. The fecal samples were collected by using a toothpick, avoiding urate deposits and were placed in plastic vials containing 2.5% (w/v) potassium dichromate solution ($K_2Cr_2O_7$) at a ratio of 1:4 (v/v). Samples were placed in a thin layer (c.5 mm) of $K_2Cr_2O_7$ 2.5% solution in Petri dishes, incubated at 20–26°C and monitored daily under a light microscope (Duszynski & Wilber, 1997). Oöcysts (n = 30) were microscopically examined using the technique described by Duszynski & Wilber (1997) and Berto et al. (2014a). Morphological observations, photomicrographs and measurements were made using a Nikon Eclipse 80i binocular microscope (Nikon Corporation, Tokyo, Japan)

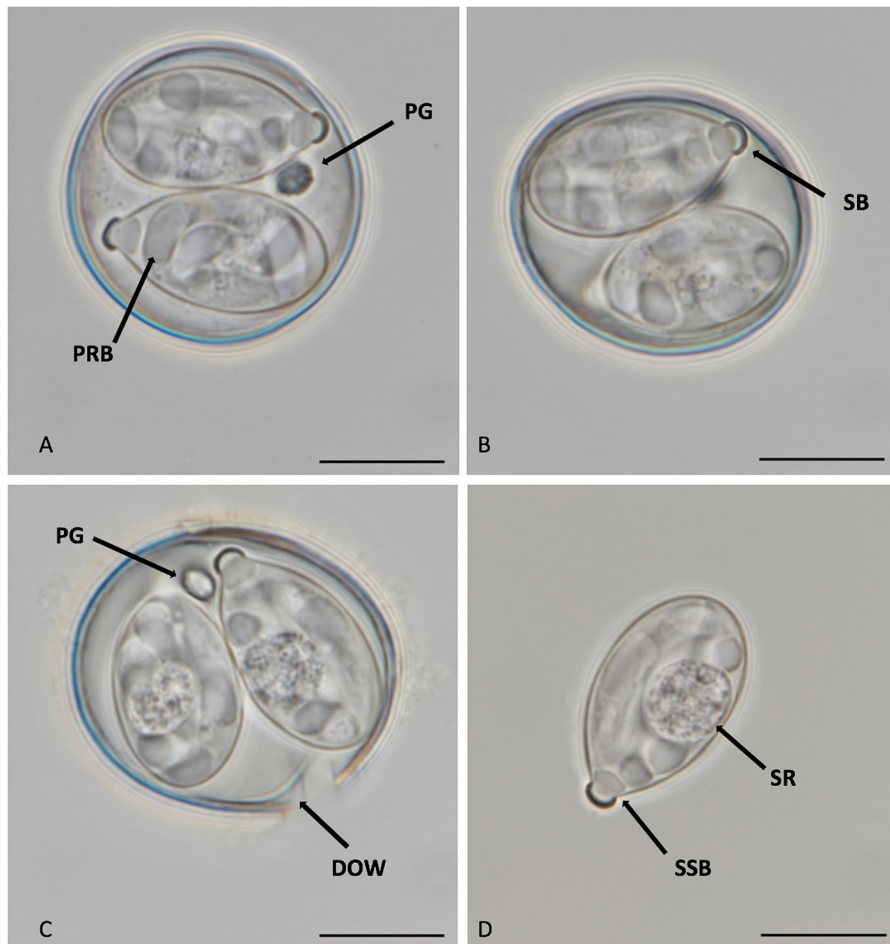


Fig. 1 Photomicrographs of sporulated oöcysts and sporocysts of *Isospora speciosae* n. sp. **A**, Subspherical oöcyst with clearly visible ovoidal sporocysts with a polar granule (PG) between them. PRB, posterior refractile body of the sporozoite; **B**, Two sporocysts with clearly half-moon shaped Stieda body (SB); **C**, One fractured oöcyst with clearly visible double outer wall (DOW); **D**, One sporocyst released from the oöcyst showing sub-Stieda body (SSB) and sporocyst residuum (SR), consisting of many spherules. Scale-bars: 10 µm.

coupled to a digital camera Nikon DS-Fi2 (Nikon Corporation, Tokyo, Japan) and a composite line drawing made. All measurements are in micrometers and are given as the range followed by the mean in parentheses.

Results

Three of the seven fecal samples examined contained oöcysts. Two days after the collection of samples, more than 70% of the oöcysts were sporulated (under the conditions used in this study).

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

Isospora speciosae n. sp.

Type-host: *Geothlypis speciosa* Sclater (Aves: Passeriformes: Parulidae), black-pollled yellowthroat.

Type-locality: Chimaliapan marsh (DOF, 2018), Ciénegas del Lerma (19°20′24″N, 99°29′26″W; 19°21′28″N, 99°31′10″W), State of Mexico, Mexico.

Type-material: Oöcysts in dichromate solution, phototypes and line drawings of sporulated oöcysts are deposited and available in the Repository (www.ibirds.org) of the Institute for Biodiversity Research, Development & Sustainability (iBIRDS). Photographs of the type-host specimens (symbiotypes) are deposited in the same collection. Photomicrographs of sporulated oöcysts are deposited and available in the Repository of iBIRDS (www.ibirds.org). The repository number is ESV-29/2022.

Prevalence: Oöcysts of this species were found in 3/7 (42%) of the fresh faecal samples examined.

Site of infection: Unknown. Oöcysts were recovered from faeces.

ZooBank registration: To comply with 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 speciosa* is urn:lsid:zoobank.org:pub:96F2C64C-35E5-4592-BD64-7B057A94DF08.

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

Description (Figs. 1, 2).

Sporulated oöcyst

Oöcysts (n = 30) subspherical to ovoidal, 23–28 × 13–27 (26.6 × 25.4). Wall bi-layered, 1.2–1.4 (1.3), outer layer smooth, 1/3 of total thickness; length/width (L/W) ratio 1.0–1.1 (1.1). Micropyle and oöcyst residuum absent. Polar granule present, 1 or 2 (2.0 × 4.0) (Figs. 1, 2).

Sporocyst and sporozoites

Sporocyst (n = 30) are ovoidal, 17–19 × 9–11 (18.7 × 10.2); length/width (L/W) ratio 1.7–1.8 (1.8). Stieda body present, half-moon-shaped (0.5 thick); sub-Stieda body present, trapezoidal, prominent, 1.7 high × 3.0 wide; para-Stieda body absent. Sporocyst residuum present, consisting of many spherules (0.3–0.6) (Fig. 2C). Sporozoites 4, vermiform, 3.3–3.6 × 15.0–16.0, with posterior refractile body (5.5 in length), anterior refractile body (2.8 in diameter) and indiscernible nucleus. Discrete striations (3–5) are present between the posterior refractile body and anterior refractile body (Figs. 1, 2).

Remarks

Seven parulid species have been reported as host of *Isospora* spp.: *Basileuterus culicivorus* (Deppe) for *I.*

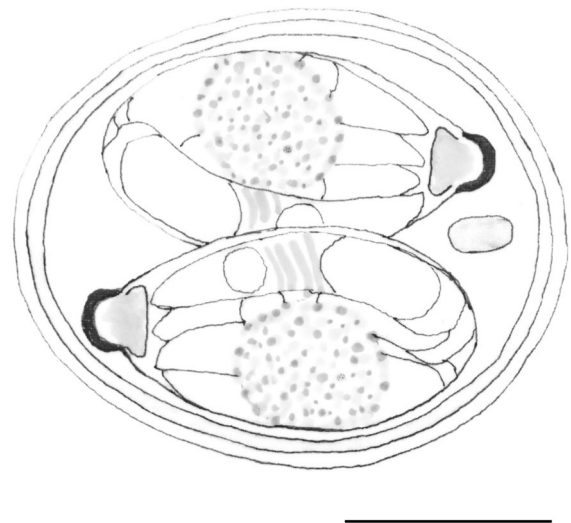


Fig. 2 Line drawing of a sporulated oöcyst of *Isospora speciosae* n. sp. from *Geothlypis speciosa*. Scale-bar: 10 μ m.

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

Species	<i>Isoospora speciosae</i> n. sp.	<i>Isoospora basileuterusi</i>	<i>Isoospora cardellinae</i>	<i>Isoospora celata</i>	<i>Isoospora piacobrai</i>	<i>Isoospora orbisreinitas</i> Keeler, Yabsley, Adams & Hernandez, 2014
Locality	Mexico	Brazil	Mexico	Mexico	Brazil	Costa Rica
Host	<i>Geothlypis speciosa</i> subsp. <i>speciosa</i> (Sclater)	<i>Basileuterus culicivorus</i> (Deppe)	<i>Cardellina rubra</i> (Swainson)	<i>Leiothlypis celata</i> (Say)	<i>Geothlypis aequinoctialis</i> (Gmelin)	<i>Basileuterus rufifrons</i> (Swainson)
Reference	Present study	Mello et al. (2022)	Salgado-Miranda et al. (2016)	Berto et al. (2014b)	Berto et al. (2010)	Keeler et al. (2014)
Oöcyst						
Shape	subspherical to ovoidal	ellipsoidal to ovoidal	subspherical	subspherical	subspherical to ovoidal	spherical to ovoidal
Length	24–26 (25.7)	22–28 (25.2)	23–28 (26.6)	27–30 (28.4)	21–26 (23.5)	21–28 (24.3)
Width	21–23 (22.2)	17–23 (21.1)	23–27 (25.4)	25–28 (26.4)	20–24 (21.6)	19–25 (22.3)
Length/width ratio	1.1–1.1 (1.1)	1.1–1.3 (1.2)	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	present, 1-2	present, 1-3	absent	absent	present, 1	present, 0-4, spherical to cigar-shaped
Oöcyst	residium	absent	absent	absent	present, compact mass	absent
absent Sporocyst						
Shape	ovoidal	ellipsoidal to lemon-shaped	ovoidal	ovoidal	ovoidal	ovoidal
Length	17–19 (18.7)	14–17 (15.3)	18–20 (19.0)	15–20 (18.2)	15–17 (15.8)	12–19 (16.0)
Width	9–11 (10.2)	8–11 (9.5)	11–13 (12.0)	11–14 (12.8)	9–12 (10.5)	10–14 (11.8)
Length/width ratio	1.7–1.8 (1.8)	1.4–1.8 (1.61)	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	half-moon-shaped	knob-like	knob-like	knob-like	prominent; knob-like	knob-like
Substieda body	trapezoidal	trapezoidal	trapezoidal, irregular base	irregular; barely discernible	large; trapezoidal; homogeneous	prominent; trapezoidal; compartmentalized
Residium	granules, compact	granules membrane-bound	diffuse	diffuse	diffuse	diffuse

Table 1 continued

Species	<i>Isospora speciosae</i> n. sp.	<i>Isospora basileuterusi</i> Mello, Oliveira, Andrade, Cardozo, Oliveira, Lima & Berto, 2022	<i>Isospora cardellinae</i> Salgado-Miranda, Medina, Zepeda-Velázquez, García-Conejo, Galindo-Sánchez, Janczur & Soriano-Vargas, 2016	<i>Isospora celata</i> Berto, Medina, Salgado-Miranda, García-Conejo, Janczur, Lopes & Soriano-Vargas, 2014	<i>Isospora piacobrai</i> Berto, Flausino, Luz, Ferreira & Lopes, 2010	<i>Isospora orbisreinitas</i> Keeler, Yabsley, Adams & Hernandez, 2014
Locality	Mexico	Brazil	Mexico	Mexico	Brazil	Costa Rica
Host	<i>Geothlypis speciosa</i> subsp. <i>speciosa</i> (Sclater)	<i>Basileuterus culicivorus</i> (Deppe)	<i>Cardellina rubra</i> (Swainson)	<i>Leiothlypis celata</i> (Say)	<i>Geothlypis aequinoctialis</i> (Gmelin)	<i>Basileuterus rufifrons</i> (Swainson)
Reference	Present study	Mello et al. (2022)	Salgado-Miranda et al. (2016)	Berto et al. (2014b)	Berto et al. (2010)	Keeler et al. (2014)
Sporozoite						
Length	15–16					
Width	3.3–3.6					
Striations	present 3-5	absent	absent	absent	absent	absent

basileuterusi (see Mello et al., 2022), *Cardellina rubra* (Swainson) for *I. cardellinae* (see Salgado-Miranda et al., 2016), *Leiothlypis celata* (Say) for *I. celata* (see Berto et al., 2014b), *Basileuterus rufifrons* (Swainson) for *I. orbisreinitas* (see Keeler et al., 2014), and *Geothlypis aequinoctialis* (Gmelin) for *I. piacobrai* (see Berto et al., 2010). The common yellowthroat *Geothlypis trichas* (Linnaeus) (see Boughton et al., 1938) and the Nashville warbler *Leiothlypis ruficapilla* (Wilson) (see Swayne et al., 1991), host for an undescribed isosporoid coccidia. The morphology and morphometry of the oöcysts of *I. speciosae* allow differentiating it from the other *Isospora* species reported (Table 1). The mean dimensions of the sporulated oöcysts (25.7×22.2) in *I. speciosae* n. sp. appear to be considerably smaller than those in *I. celata* (28.4×26.4). In *I. speciosae* the Stieda body is prominent and half-moon-shaped while knob-like in *I. basileuterusi*, *I. cardellinae*, *I. celata*, *I. orbisreinitas* and *I. piacobrai*. A polar granule is absent in *I. cardellinae* and *I. celata* (Table 1). Striations (3-5) are only present in *I. speciosae* (Figs. 1, 2; Table 1).

Discussion

To date, no helminth or protist parasites have been described in *G. speciosa*. Of the 115 warbler species that occur in the New World, only seven have been reported as hosts of *Isospora* spp. as mentioned above. This low frequency may not reflect the distribution and prevalence of *Isospora* in the New World warblers but is rather an outcome of a small number of studies on the genus *Isospora* from Parulidae (Berto & Lopes, 2013).

The sporulated oöcysts 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 (Table 1) (Duszynski & Wilber, 1997; Berto et al., 2014b). In conclusion, *I. speciosae* is considered as a species new to science, being the sixth species of the genus described from a New World parulid species.

Acknowledgements Both Comités Ejidales de Capulhuac de Miramontes and San Pedro Tultepec, Mexico, are gratefully acknowledged by access permissions to Ciénegas del Lerma marshes.

Author contributions This study was designed by both authors. Field collections were performed by both authors. Laboratory procedures for maintenance, recovery,

measurements, photomicrographs and isolation of oöcysts were performed by CSM. ESV drew the coccidian oöcyst. The manuscript was written by both authors.

Funding This study was funded by Institute for Biodiversity Research, Development & Sustainability (iBIRDS, Mexico).

Data availability Photosyntypes, line drawing, and oöcysts in 70% ethanol are deposited and available (www.ibirds.org) in the Repository of the iBIRDS, under the repository number ESV-29/2022, along with the photographs of the type-host specimen (symbiotype). Also, photographs of the type-host specimen are available at the Macauley Library (accession numbers ML310666731, ML363566601, ML295909441, ML290324271, ML290527531, ML295892161, and ML290515461).

Declarations

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.

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