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Brief report: *Isospora gilvusi* n. sp. (Apicomplexa: Eimeriidae) from the warbling vireo *Vireo gilvus* Vieillot (Passeriformes: Vireonidae) in Morelia, Mexico

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Abstract A new coccidian species, *Isospora gilvusi* **n. sp.** (Apicomplexa: Eimeriidae) collected from the warbling vireo *Vireo gilvus*, is reported from Morelia, Michoacán State, Mexico. Sporulated oöcysts of the new species are spherical to subspherical, 27−31 × 27−29 (30.1 × 28.4) 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. Sporocyts are ovoid to drop-shaped, 16−17 × 11−12 (17.0 × 11.5) µm, with a L/W ratio of 1.7; Stieda and sub-Stieda bodies are both present, but para-Stieda body is absent; sporocyst residuum diffuse. At the histological study, endogenous stages were observed in the epithelial cells of the duodenum. This is the second species of *Isospora* recorded infecting a bird of the family Vireonidae in the New World.

Introduction

The warbling vireo *Vireo gilvus* Vieillot occupies a variety of deciduous forest habitats, predominantly riparian, of the Americas. The warbling vireo appears well adapted to human landscapes, as nests have been found in neighbourhoods, urban parks, orchards, and farm fencerows (Gardali & Ballard, 2020).

Coccidians are obligate intracellular parasitic chromists (Ruggiero et al., 2015). Species infecting birds are homoxenous and are thought to be reasonably host-specific (Tenter et al., 2002). To date, only one coccidian species, *Isospora pitiguari* Lopes et al. (2014), has been described from a bird species of the family Vireonidae, the rufous-browed peppershrike *Cyclarhis gujanensis* Gmelin in Brazil (Lopes et al., 2014). An undescribed isosporoid coccidia has been reported in the red-eyed vireo *Vireo olivaceus* in USA (Boughton et al., 1938). Hence, the aim of the present study was the description of a new *Isospora* species infecting the warbling vireo *V. gilvus* in Morelia, Mexico.

Materials and methods

A male, adult warbling vireo (*Vireo gilvus*) was killed by a domestic cat in a backyard at Morelia City (19° 40′ 41″ N, 101° 10′ 23″ W), Mexico. Faecal samples were collected from the small intestine 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

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described by Duszynski & Wilber (1997) and Berto et al. (2014). Morphological observations, photomicrographs and measurements were made using a Nikon Eclipse 80*i* binocular microscope (Nikon Corporation, Tokyo, Japan) 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.

The bird was preserved in 10% neutral buffered formalin and processed, sectioned, and stained with hematoxylin and eosin for routine histological examination as previously reported (Salgado-Miranda et al., 2016). The following organs and tissues were sectioned: lungs, esophagus, proventriculus, ventriculus, duodenum, small intestine, and liver. Photomicrographs of slides were made using microscope above described.

Results

The faecal sample examined contained oöcysts. Two days after the collection of the sample, more than 70% of the oöcysts were sporulated (under the conditions used in this study).

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

Isospora gilvusi n. sp.

Type-host: *Vireo gilvus* Vieillot (Aves: Passeriformes: Vireonidae), warbling vireo.
Type-locality: Área Natural Protegida La Loma de Santa María (19° 40′ 41″ N, 101° 10′ 23″ W), Morelia, State of Michoacán, 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-31/2023.
Prevalence: Oöcysts of this species were found in 1/1 (100%) of the fresh faecal samples examined.
Site of infection: Duodenum.
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 gilvusi* is urn:lsid:zoobank.org:act:6DA52C6D-3C96-4BAA-BB2E-559E53E96AA5.
Etymology: The specific name is derived from the species name of the type-host.

Description (Figs. 1 and 2).

Sporulated oöcyst

Oöcysts (n = 30) spherical to subspherical, 27–31 × 27−29 (30.1 × 28.4). Wall bi-layered, 1.2−1.5 (1.4), 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 (2.2 × 2.8) (Figs. 1 and 2).

Sporocyst and sporozoites

Sporocyst (n = 30) are ovoid to drop-shaped, 16–17 × 11–12 (17.0 × 12.0); length/width (L/W) ratio 1.6–1.8 (1.7). Stieda body present, nipple-like shape; sub-Stieda body present, rounded, 2.5 high × 3.5 wide; para-Stieda body absent. Sporocyst residuum present, diffuse, consisting of many spherules (0.3–0.6) (Fig. 2C). Sporozoites 4, vermiform, 15.0–16.0 × 3.2–3.4 (15.5 × 3.3), with posterior refractile body (5.1 in length), anterior refractile body (2.7 in diameter) and indiscernible nucleus (Figs. 1 and 2).

Endogenous forms

Histopathological examination of tissues helped detect endogenous stages in the epithelial cells of the duodenum. Endogenous stages develop extranuclearly in the cytoplasm of epithelial cells. Most of the meronts were observed into epithelial cells of the crypts (Fig. 3).

Remarks

Two Vireonidae species have been reported as host of *Isospora* spp.: *Vireo olivaceus* (Linnaeus) for an

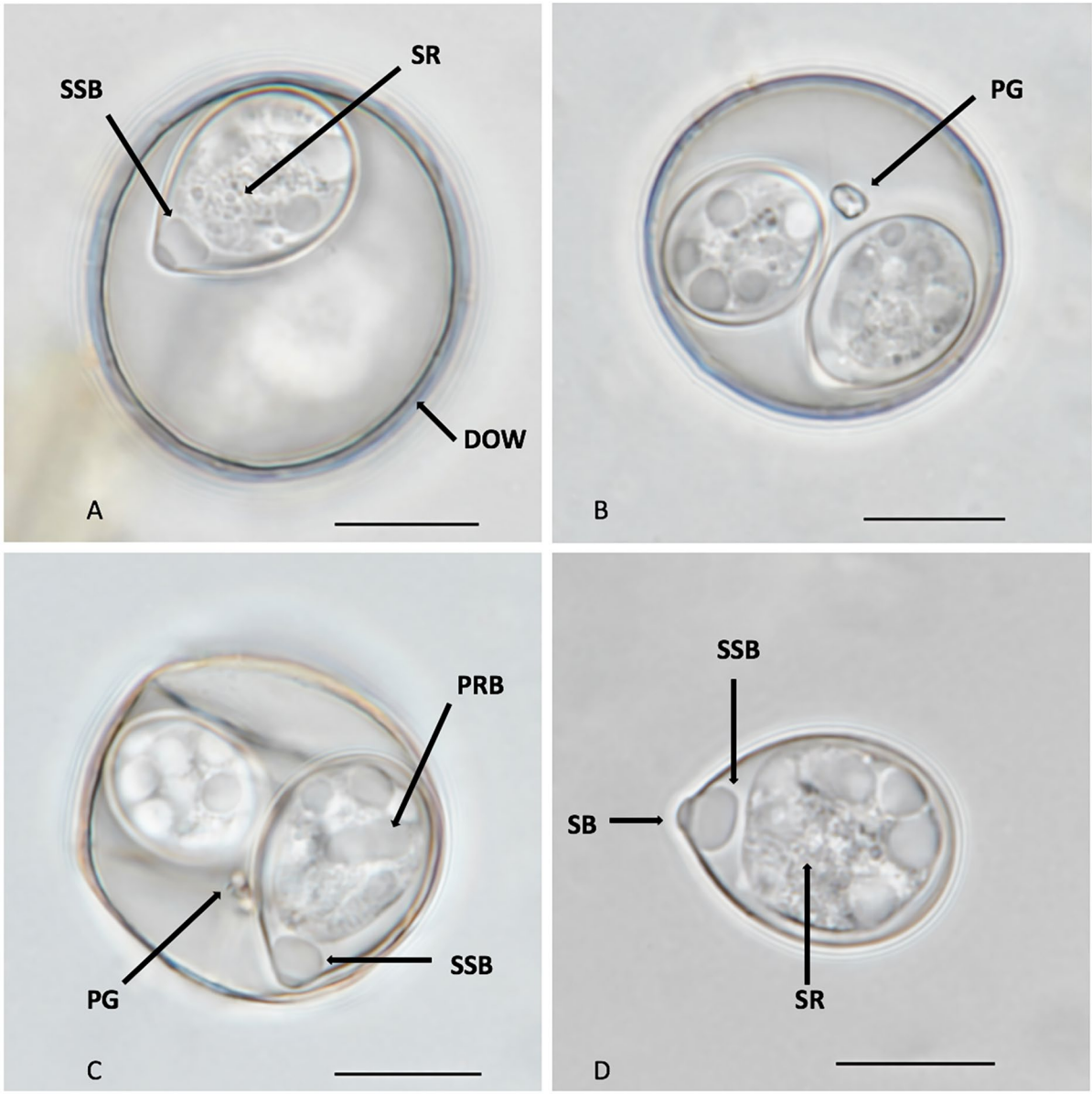

Fig. 1 Photomicrographs of sporulated oöcysts and sporocysts of *Isospora gilvusi* **n. sp. A** Subspherical oöcyst with an ovoid to drop-shaped sporocyst. DOW, double-layered outer wall, SSB, sub-Stieda body, and SR, sporocyst residuum. **B** Subspherical oöcyst with two sporocysts with clearly visible polar granule (PG); **C** one fractured oöcyst with clearly visible sub-Stieda body (SSB), posterior refractile body (PRB) and polar granule (PG); **D** one sporocyst released from the oöcyst showing Stieda body (SB), rounded sub-Stieda body (SSB) and diffuse sporocyst residuum (SR), consisting of many spherules. *Scale-bars*: 10 µm.

undescribed *Isospora* species (see Boughton et al., 1938) and *Cyclarhis gujanensis* (Gmelin) for *I. pitiguari* (see Lopes et al., 2014). The morphology and morphometry of the oöcysts of *I. gilvusi* allow differentiating it from *Isospora pitiguari*. The mean dimensions of the sporulated oöcysts (30.1 × 28.4) in *I. gilvusi* **n. sp.** appear to be considerably larger than those in *I. pitiguari* (26.8 × 25.7). A polar granule is absent in *I. pitiguari*. In *I. gilvusi* the Stieda body is nipple-like while knob-like in *I. pitiguari*. The

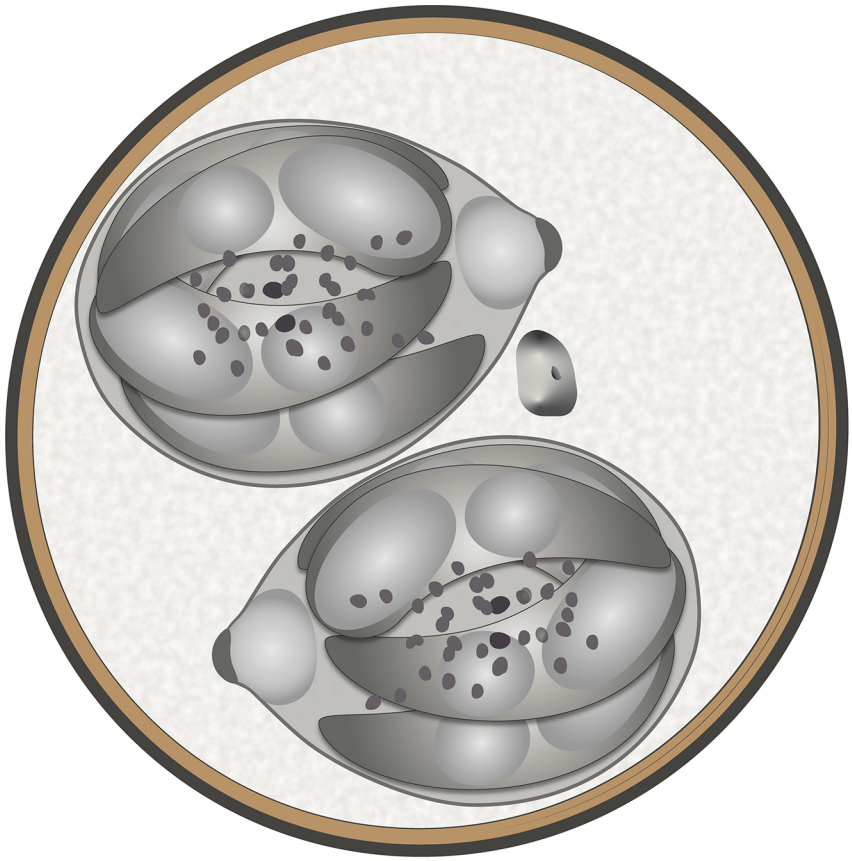

Fig. 2 Line drawing of a sporulated oöcyst of *Isospora gilvusi* **n. sp.** from *Vireo gilvus*. *Scale-bar*: 10 µm.

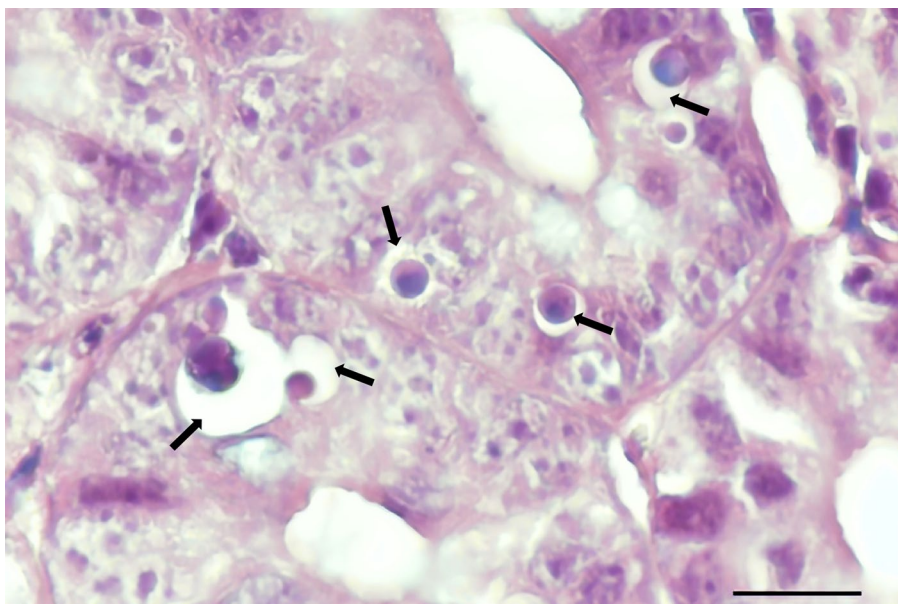

Fig. 3 Histological sections of a naturally coccidian-infected warbling vireo *Vireo gilvus*. Duodenum, meronts of *Isospora gilvusi* **n. sp.** surrounded by its parasitophorous vacuole (arrows).

morphometry of the sporocysts of *I. gilvusi* (ovoid to drop-shaped) allow differentiating it from *Isospora pitiguari* (rounded to slightly ovoidal). The length of the sporocysts (17.0) in *I. gilvusi* **n. sp.** appear to be larger than those in *I. pitiguari* (14.4).

Discussion

Domestic cats are a potential risk for wildlife in the Neotropics. Both native and non-native birds are among the main preys captured by domestic cats in the City of Xalapa, Veracruz, Mexico (Mella-Méndez et al., 2022).

To date, not helminth or protist parasites have been described in *V. gilvus*. Of the 37 Vireonidae species that occur in the New World, only two have been reported as host of *Isospora* spp. This reflect the small number of studies on the genus *Isospora* from Vireonidae (Berto & Lopes, 2013).

Five *V. gilvus* subspecies are divided into two groups: Eastern group, including *V. gilvus* subsp. *gilvus*, and Western group, including *V. gilvus* subsp. *swainsoni*, *V. gilvus* subsp. *victoriae*, *V. gilvus* subsp. *brewsteri*, and *V. gilvus* subsp. *sympatricus* (Gardali & Ballard, 2020). According with geographic distribution, the bird included in the present study belongs to *V. gilvus* subsp. *brewsteri*. Geographic range or distribution of *V. gilvus* subsp. *gilvus* is sympatric with *C. gujanensis* (Brewer et al., 2020). Studies on coccidians from those sympatric populations are needed to determine possible cross infections with *I. gilvusi* and *I. pitiguari*. As *I. gilvusi* is morphologically similar to *I. pitiguari*, our study contributes to the knowledge of coccidians that infect New World Vireonidae species, particularly the several subspecies of the warbling vireo *V. gilvus*. Furthermore, histological studies are needed to determine tissue tropism of coccidians that infect those vireos.

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 (Duszynski & Wilber, 1997; Berto et al., 2014). The histopathological study demonstrated the *Isospora* intestinal infection, in which various meronts were observed, in the warbling vireo *V. gilvus*. In conclusion, *I. gilvusi* is considered as a species new to science, being the second species of the genus infecting a New World Vireonidae species.

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Author contributions This study was performed by both authors. Laboratory procedures for maintenance, recovery, measurements, photomicrographs and isolation of oöcysts were performed by both authors. CGL drew the coccidian oöcyst. The manuscript was written by both authors.

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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-31/2023, along with the photographs of the type-host specimen (symbiotype). Also, a photograph of the type-host specimen are available at the Macauley Library (accession number ML579487331).

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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