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Three new species of Isospora Schneider, 1881 (Apicomplexa: Eimeriidae) infecting the song sparrow Melospiza melodia Wilson, 1810 (Aves: Passeriformes: Passerellidae) in Toluca Valley, Mexico

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Three new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) infecting the song sparrow *Melospiza melodia* Wilson, 1810 (Aves: Passeriformes: Passerellidae) in Toluca Valley, Mexico

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Abstract Three new isosporan (Apicomplexa: Eimeriidae) species from the song sparrow *Melospiza melodia* Wilson in Mexico are described in the present study. The sporulated oöcysts of *Isospora cantoris* n. sp. are subspherical, 19.4–21.5 × 16.9–18.9 (20.5 × 18.0), with a length/width (L/W) radio 1.0–1.2 (1.04), mycropile and oöcyst residuum absent and one compact polar granule present. The sporulated oöcysts of *Isospora melodiae* n. sp. are spherical to subspherical, 20.2–21.4 × 18.5–19.7 (20.8 × 19.1), with a length/width (L/W) radio 1.0–1.1 (1.05), mycropile and oöcyst residuum absent and one compact polar granule present. The sporulated oöcysts of *Isospora villai* n. sp. are subspherical, 20.7–23.8 × 19.4–23.3 (22.3 × 21.4), with a length/width (L/W) radio 1.0–1.2 (1.1), mycropile, oöcyst residuum and polar granule absent. In conclusion, *I. cantoris*, I. *melodiae* and *I. villai* are coccidian species new to science, being a total of seven species of the genus described from New World sparrows.

Introduction

The song sparrow *Melospiza melodia* Wilson (Passeriformes: Passerillidae), is one of the most diverse and widespread songbirds in North America, with 25 diagnosable subspecies breeding from Newfoundland to the Aleutian Islands of Alaska and south to central Mexico (Arcese et al., 2020). Females build and incubate open-cup nests, wherever suitable cover and insect food are present. Males are recognized everywhere by their distinct melodic song (Arcese et al., 2020). The song sparrow is one of the most common and widespread sparrows in Toluca Valley, Mexico (Soriano-Vargas, 2016).

Infectious microorganisms identified from the song sparrow include *Plasmodium homopolare* (Carlson et al., 2018), *Parahaemoproteus* spp. (Vaziri et al., 2019), *Haemoproteus fringillae* (Martinsen et al., 2008), and unidentified cestodes, nematodes and acanthocephalans (Vaziri et al., 2019). Up to now, three species of *Isospora* have been identified in Passerillidae: *I. andesensis* in the common chlorospingus (*Chlorospingus flavopectus*) (Templar et al., 2004), *I. ticoticoi* in the rufous-collared sparrow (*Zonotrichia capensis*) (Balthazar et al., 2009) and *I. pittmani* in the dark-eyed junco (*Junco hyemalis*) (McAllister & Hnida, 2023). An undescribed isosporoid coccidia was reported in the Savannah sparrow (*Passerculus sandwichensis*) in USA (Boughton et al., 1938). A coinfection of an undescribed *Isospora* sp. and *Eimeria atlapetesi* was described in the endemic, rufous-capped brushfinch (*Atlapetes pileatus*) in Mexico (Soriano-Vargas et al., 2015; Soriano-Vargas et al., 2017). This paper describes three *Isospora* species infecting the song sparrow *M. melodia* in Ciénegas del Lerma and Bordo Las Maravillas, Toluca Valley, Mexico. The three *Isospora* species occurred within a same individual.

Materials and methods

From March 2021 to June 2025, during trips to marshes Ciénegas del Lerma (2600 m a.s.l., 19°20'24"N, 99°29'26"W; 19°21'28"N, 99°31'10"W) and the marsh Bordo Las Maravillas at the Campus El Cerrillo (2607 m a.s.l., 19°17'32"N, 99°39'14"W), Universidad Autónoma del Estado de México and in the town of San Felipe Tlalmimilolpan, State of Mexico, Mexico (2740 m a.s.l., 19°13'58"N, 99°38'45"W), song sparrows (*M. melodia* subsp. *villai*) were observed and photographed. Birds were observed eating and moving along the roads, on California bulrush (*Schoenoplectus californicus*), reed (*Thypha* sp.) and weeping willow (*Salix babylonica*). A total of 35 fresh droppings 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). The fresh faecal 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 = 90) were microscopically examined using the technique 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). A composite line drawing was made using Adobe Illustrator CC2022 and Adobe Photoshop CC2022 programs. All measurements are in micrometers and are given as the range followed by the mean in parentheses.

On September 24, 2022, one moribund bird was found, euthanised by method recommended by AVMA (2020) and necropsied. Also, a faecal sample was collected. 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 included: lungs, oesophagus, proventriculus, ventriculus, duodenum, small intestine, and liver. Photomicrographs of slides were made using microscope above described.

Results

Fourteen (38.8%) of the 36 faecal 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). Three morphometrical, distinguishable types of oöcysts were observed. Three faecal samples contained two types of oöcysts, and two faecal samples contained the three types of oöcysts.

Family Eimeriidae Minchin, 1903

Genus *Isospora* Schneider, 1881

***Isospora cantoris* n. sp.**

Type-host: *Melospiza melodia* Wilson, 1810 (Aves: Passeriformes: Passerellidae).

Type-locality: Marshes 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. The repository number is ESV-34/2026.

Prevalence: Oöcysts of this species were found in 5/36 (13.8%) of the faecal samples examined.

Site of infection. Duodenum or small intestine.

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 cantoris* is urn:lsid:zoobank.org:act: A80BEE1D-8D9F-4CCC-A36E-D7B56F640D20.

Etymology: The specific name is derived from the Spanish name of the type-host, *gorrión cantor* (song sparrow; singer).

Description (Figs. 1A, 1B, 2A).

Sporulated oöcyst

Oöcysts (n = 30) subspherical, 19.4–21.5 × 16.9–18.9 (20.5 × 18.0). Wall double layered (*c*. 0.9 thick), outer layer smooth was 2/3 of total thickness; length/width (L/W) radio 1.0–1.2 (1.04). Mycropile and oöcyst residuum absent. Polar granule present, one compact (Figs. 1A, 1B, 2A).

Sporocyst and sporozoites

Sporocyst (n = 30) are ovoidal, 13.5–14.5 × 9.0–9.8 (14.1 × 9.4); length/width (L/W) radio 1.4–1.5 (1.5). Stieda body present, nipple-like; subStieda body present, rounded; para-Stieda body absent. Sporocyst residuum present, consisting of compact granules (Fig. 1B). Sporozoites 4, vermiform, 12.8–13.0 × 2.9–3.2 (12.9 × 3.0), with posterior refractile body (3.4 in length), anterior refractile body (2.5 in diameter) and indiscernible nucleus.

***Isospora melodiae* n. sp.**

Type-host: *Melospiza melodia* Wilson, 1810 (Aves: Passeriformes: Passerellidae).

Type-locality: Marshes 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. The repository number is ESV-35/2026.

Prevalence: Oöcysts of this species were found in 5/36 (13.8%) of the faecal samples examined.

Site of infection. Duodenum or small intestine.

Zoobank registration: The Life Science Identifier (LSID) for *Isospora melodiae* is urn:lsid:zoobank.org:act: F2A58D79-2DCD-4B6E-B34B-39A8F90AD0F4.

Description (Figs. 1C, 1D, 2B).

Sporulated oöcyst

Oöcysts (n = 30) spherical to subspherical, 20.2–21.4 × 18.5–19.7 (20.8 × 19.1). Wall double layered (*c*. 1.0 thick), outer layer smooth was 2/3 of total thickness; length/width (L/W) radio 1.0–1.1 (1.05). Mycropile and oöcyst residuum absent. Polar granule present, one compact (Figs. 1C, 1D, 2B).

Sporocyst and sporozoites

Sporocyst (n = 30) are ovoidal, 13.2–14.8 × 9.2–9.8 (14.0 × 9.5); length/width (L/W) radio 1.4–1.5 (1.4). Stieda body present, nipple-like; subStieda body present, trapezoidal; para-Stieda body absent. Sporocyst residuum present, consisting of few granules (Fig. 1D). Sporozoites 4, vermiform, 12.7–12.9 × 2.8–3.0 (12.8 × 2.9), with posterior refractile body (4.5 in length), anterior refractile body (2.7 in diameter) and indiscernible nucleus.

***Isospora villai* n. sp.**

Type-host: *Melospiza melodia* Wilson, 1810 (Aves: Passeriformes: Passerellidae).

Type-locality: Marshes 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. The repository number is ESV-36/2026.

Prevalence: Oöcysts of this species were found in 4/36 (11.1%) of the faecal samples examined.

Site of infection. Duodenum or small intestine.

Zoobank registration: The Life Science Identifier (LSID) for *Isospora villai* is urn:lsid:zoobank.org:act: 8B3E95C8-1263-4C08-8E17-74AA9A28924F.

Description (Figs. 1E, 1F, 2C).

Sporulated oöcyst

Oöcysts (n = 30) subspherical, 20.7–23.8 × 19.4–23.3 (22.3 × 21.4). Wall double layered (*c*. 1.1 thick), outer layer smooth was 3/4 of total thickness; length/width (L/W) radio 1.0–1.2 (1.1). Mycropile, oöcyst residuum and polar granule absent (Figs. 1E, 1F, 2C).

Sporocyst and sporozoites

Sporocyst (n = 30) are ellipsoidal, 16.1–17.7 × 10.1–10.8 (17.1 × 10.4); length/width (L/W) radio 1.6–1.7 (1.6). Stieda body present, rounded; subStieda body present, rounded; para-Stieda body absent. Sporocyst residuum present, consisting of diffuse granules (Fig. 1F). Sporozoites 4, vermiform, 15.4–15.8 × 3.2–3.4 (15.6 × 3.3), with posterior refractile body (4.7 in length), anterior refractile body (2.7 in diameter) and indiscernible nucleus.

In the histological study, conspicuous meronts surrounded by its parasitophorous vacuole were observed in duodenum and small intestine of the naturally isosporan-infected song sparrow *Melospiza melodia* included in the study (Fig. 3).

Remarks

Five passerellid species have been reported as host of *Isospora* spp.: *Chlorospingus flavopectus* (de Lafresnaye) for *I. andesensis* (see Templar et al., 2004), *Zonotrichia capensis* (Müller) for *I. ticoticoi* (see Balthazar et al., 2009), *Junco hyemalis* (Linnaeus) for *I. pittmani* (see McAllister & Hnida, 2023), and two undescribed *Isospora* sp. in *Passerculus sandwichensis* (Gmelin) and *Atlapetes pileatus* (Wagler) (see Boughton et al., 1938; Soriano et al., 2017). When the new species are compared to the only three isosporans from other members of the family (Table 1), its oöcysts

and sporocysts are similar in size to them. However, significant morphological differences exist between these and the three new species. The sporocyst of *I. pittmani* contains a barely discernible SSB (see McAllister & Hnida, 2023), while SSB is absent in *I. andesensis* (see Templar et al., 2004) and PG is absent in *I. ticoticoi* (see Balthazar et al., 2009).

Discussion

Of the 138 Passerellidae species that occur in the New World, only three have been reported as host of *Isospora* spp. as mentioned above. This low frequency may not reflect the distribution and prevalence of *Isospora* in the New World sparrows but is rather an outcome of a small number of studies on the genus *Isospora* from Passerellidae (Berto & Lopes, 2013).

The sporulated oöcysts obtained in this study were compared in detail with coccidian parasites from other New World sparrows that are feature-similar and belong to the same host family (Table 1) (Duszynski & Wilber, 1997; Berto et al., 2014). In conclusion, *I. cantoris*, *I. melodiae* and *I. villai* are considered as coccidian species new to science, being a total of seven species of the genus described from New World sparrows.

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Author contributions This study was designed by CSM and ESV. Field collections were performed by KPGS, UMA, CSM and ESV. Laboratory procedures for maintenance, recovery,

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

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Data availability Photosyntypes, line drawings, and oöcysts in 70% ethanol are deposited and available (www.ibirds.org) in the Repository of the iBIRDS, under the repository number ESV-30/2026, 31/2026 and 32/2026, along with the photographs of the type-host specimen (symbiotype). Also, photographs of the type-host specimen are available at the Macaulay Library (accession numbers ML299264811, ML304096411, ML356688791, and ML638302978).

Declarations

Competing interests The authors declare no competing interests.

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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Figure legends

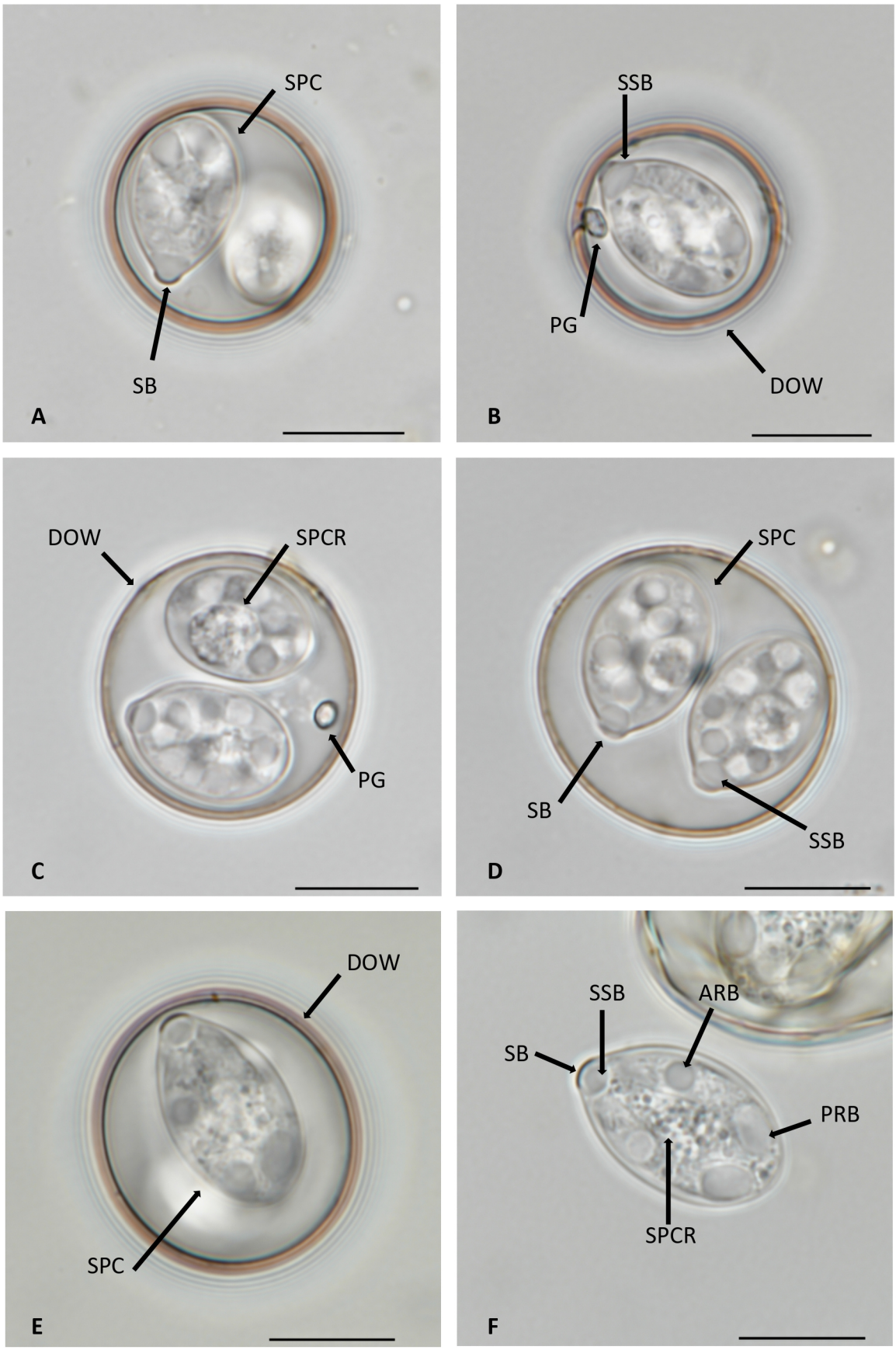
Fig. 1 Photomicrographs of sporulated oöcysts and sporocysts of new coccidian species recovered from the song sparrow *Melospiza melodia* subsp. *villai*. A, B. *Isospora cantoris* n. sp. Subspherical oöcyst with clearly visible ovoidal sporocysts with a polar granule (PG) between them. PRB, posterior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

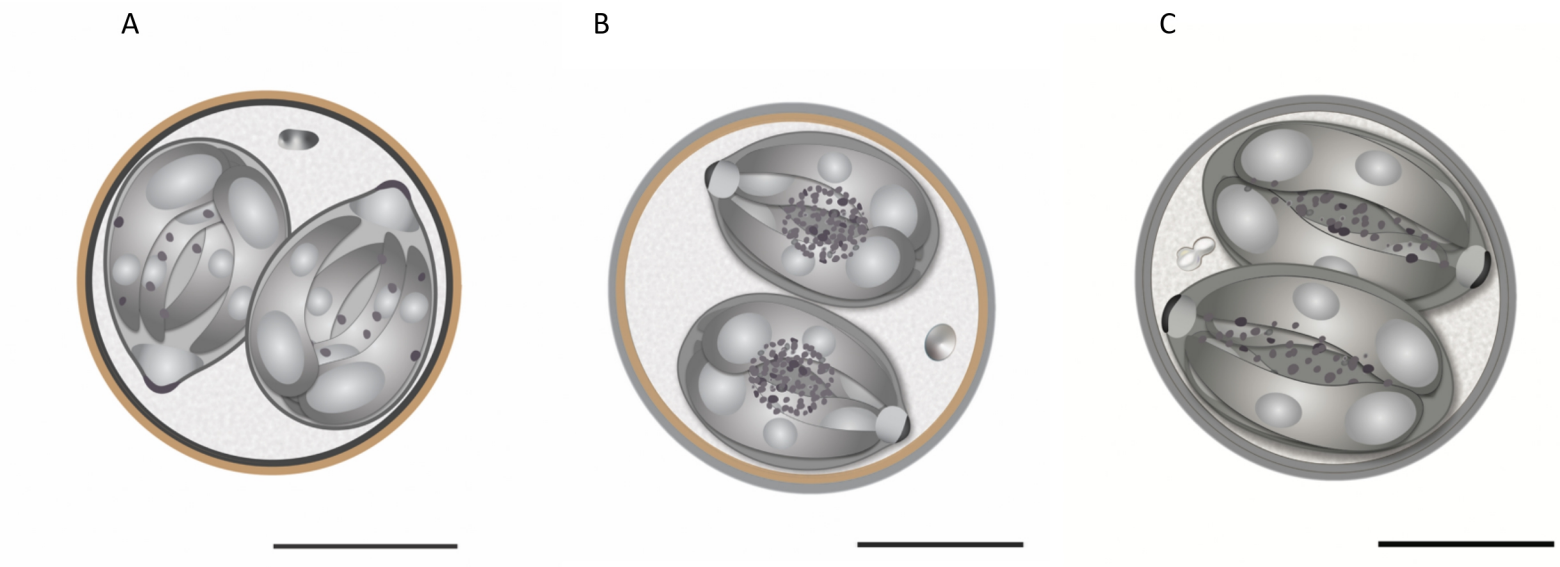
Fig. 2 Line drawings of sporulated oöcysts of new coccidian species recovered from the song sparrow *Melospiza melodia*. A. *Isospora cantoris* n. sp.; B. *I. melodiae* n. sp.; C. *I. villai* n. sp. *Scale-bar*: 10 µm.

Fig. 3 Histological section of duodenum of a naturally isosporan-infected song sparrow *Melospiza melodia* included in the study. Note conspicuous meronts surrounded by its parasitophorous vacuole (*). *Scale-bar*: 10 µm.

Table legends

Table 1. Comparative morphology of *Isospora* spp. recorded from sparrows of the New World sparrows (Passerellidae).


SPC
SB
A
SSB
PG
DOW
B
DOW
SPCR
PG
C
SPC
SB
SSB
D
DOW
SPC
E
SSB
ARB
SB
PRB
SPCR
F


A
B
C

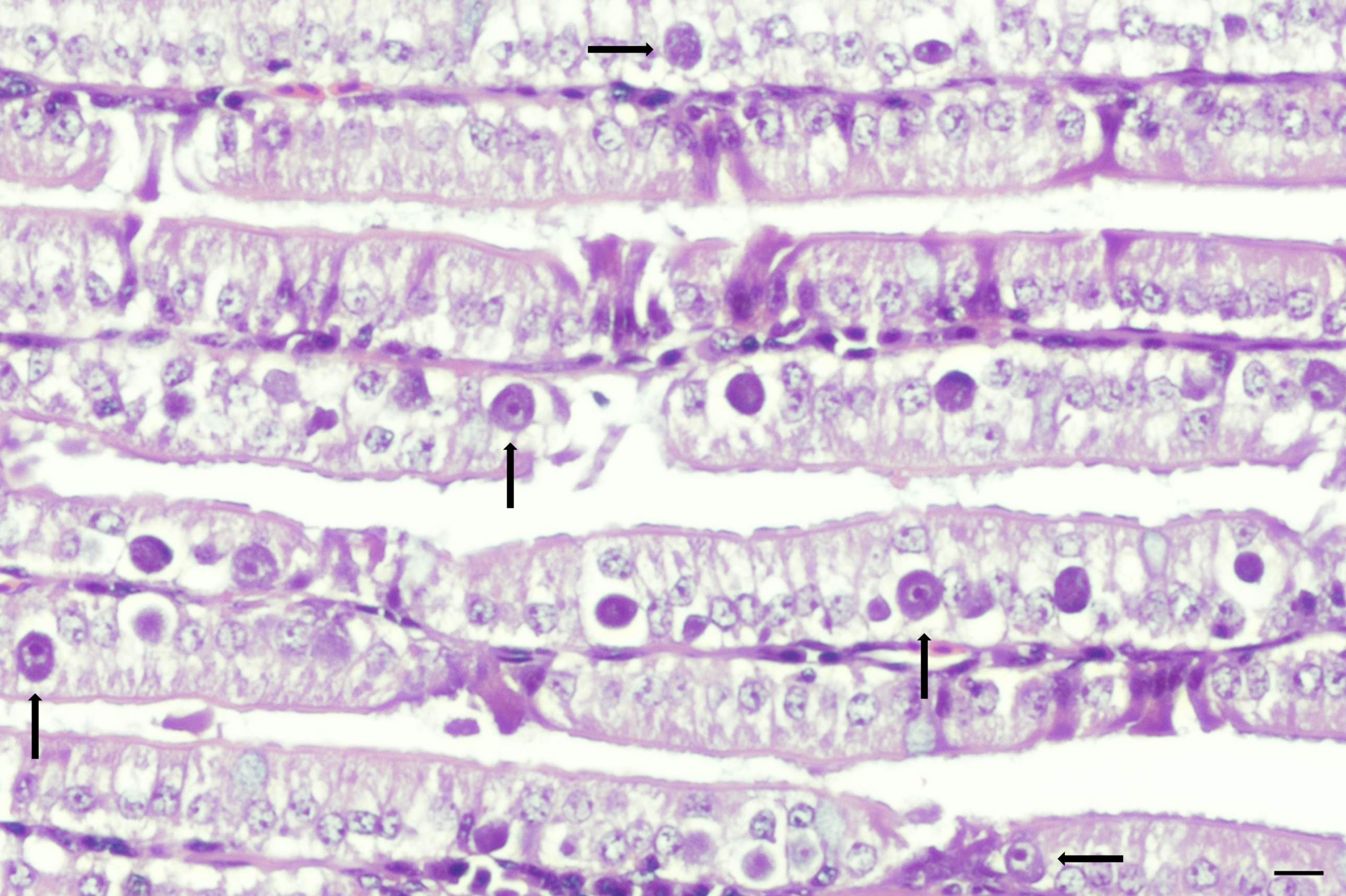


Table 1. Comparative morphology of *Isospora* spp. recorded from sparrows of the New World, Passerellidae.

Species	*Isospora cantoris* **n. sp.**	*Isospora melodiae* **n. sp.**	*Isospora villai* **n. sp.**	*Isospora andesensis* Templar, McQuistion & Capparella, 2004	*Isospora ticoticoi* Balthazar, Berto, Flausino & Lopes, 2009	*Isospora pittmani* McAllister & Hnida, 2023
Locality	Mexico	Mexico	Mexico	Peru	Brazil	USA
Host	*Melospiza melodia* (Wilson)	*Melospiza melodia* (Wilson)	*Melospiza melodia* (Wilson)	*Chlorospingus flavopectus* (de Lafresnaye)	*Zonotrichia capensis* (Müller)	*Junco hyemalis* (Linnaeus)
Reference	Present study	Present study	Present study	Templar et al. (2004)	Balthazar et al. (2009)	McAllister & Hnida (2023)
Oöcyst						
Shape	subspherical	spherical to subspherical	subspherical	subspherical to ovoidal	spherical to subspherical	spherical to ovoidal
Wall	bilayered	bilayered	bilayered	bilayered	bilayered	bilayered
Micropyle	absent	absent	absent	absent	absent	absent
Length	19.4–21.5 (20.5)	20.2–21.4 (20.8)	20.7–23.8 (22.3)	20.0–24.0 (22.6)	20.0–25.0 (23.3)	22.0–28.0 (24.8)
Width	16.9–18.9 (18.0)	18.5–19.7 (19.1)	19.4–23.3 (21.4)	17.0–20.0 (18.7)	20.0–24.0 (22.4)	21.0–27.0 (23.8)
Length/Width ratio	1.1–1.2 (1.04)	1.0–1.1 (1.05)	1.0–1.2 (1.1)	1.0–1.3 (1.2)	1.0–1.1 (1.0)	1.0–1.2 (1.04)
Polar granule	present	present	absent	present	absent	1 (typically) to 4
Oöcyst residuum	absent	absent	absent	absent	absent	absent
Sporocyst						
Shape	ovoidal	ovoidal	ellipsoidal	ovoidal	ellipsoidal	ovoidal
Length	13.5–14.5 (14.1)	13.2–14.8 (14.0)	16.1–17.7 (17.1)	13.0–15.0 (14.1)	15.0–18.0 (17.0)	13.9–17.0 (15.0)
Width	9.0–9.8 (9.4)	9.2–9.8 (9.5)	10.1–10.8 (10.4)	8.0–9.0 (8.5)	10.0–11.0 (10.8)	9.0–12.0 (10.0)
Length/Width ratio	1.4–1.5 (1.5)	1.4–1.5 (1.4)	1.6–1.7 (1.6)	1.6–1.8 (1.7)	1.5–1.7 (1.6)	1.3–1.7 (1.5)
Stieda body	nipple-like	nipple-like	rounded	triangular	nipple-like	flattened
Substieda body	rounded	trapezoidal	rounded	absent	compartmentalized	barely discernible
Residuum	granules, compact	few granules	granules, diffuse	granules, compact		granules, diffuse
Sporozoite						
Length	12.8–13.0 (12.9)	12.7–12.9 (12.8)	15.4–15.8 (15.6)			
Width	2.9–3.2 (3.0)	2.8–3.0 (2.9)	3.2–3.4 (3.3)			