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Morphological and molecular characterization of a new species of Isospora Schneider, 1881 (Apicomplexa: Eimeriidae) from the Blue Mockingbird Melanotis caerulescens in Protected Natural Area, Morelia, Michoacan, Mexico

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Research Article

Keywords: Coccidia, Isospora, Passeriform, oöcysts

Posted Date: April 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9372686/v1

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Additional Declarations: No competing interests reported.

Abstract

A new coccidian species, *Isospora melanotisi* n. sp., from the Blue Mockingbird (*Melanotis caerulescens*), from Morelia, Michoacán State, Mexico. It is described and characterized molecularly. Sporulated oöcyst of the new species are ovoid, 25–29 · 21–25 (26.9 · 23.1) µm, (n = 30), with a length/width (L/W) ratio 1.0–1.1 (1.04). Wall bi-layered 0.6–1.1 (0.8) thick, clear outer and dark interior. One polar granules present, but micropyle and oöcyst residuum are absent, Sporocysts are ovoidal, measuring on average 14–22 · 10–13 (16.6 · 10–13) µm, with a L/W ratio of 1.5; Stieda, sub-Stieda and para-Stieda bodies are present. A sporocyst residuum is present as a diffuse mass of granules. The loci segment of the gene (18S rRNA) of *I. melanotisi* n. sp. exhibited 76% genetic similarity with *I. erithaci Luscina Suecica* (MK559091.1), whit the maximum likelihood phylogenetic reconstruction method and with the Bayesian inference method, a posterior support clade of 0.8581 was formed in the *Isospora* sp. Sk01 isolate from *Sturnus vulgaris* (MN639700.1). Genetic similarity to *Isospora* sequenced with the gene loci segment (18S rRNA), morphological and molecular data support the distinct species status of the new species (Yang, 2016; Yang, 2021). To date, this is the third report on *Isospora* Schneider 1881 (Apicomplexa: Eimeriidae), which infects passerines of the Mimidae family The Americas

Introduction

The Blue Mockingbird (*Melanotis caerulescens*), a passerine bird endemic to Mexico, belongs to the Mimidae family. It is a songbird about 25 cm in length, the sexes are similar, it is characterized by its dark blue-gray plumage, darker on the ventral side, its mask of black feathers around its red eye and its black beak and legs (Peterson et al., 2008; Peterson, 2020; Berlanga et al., 2020). His behavior is withdrawn, his voice notably variable (Knight, 2018). It is located in the Trans-Mexican Neovolcanic Axis, covering states such as Jalisco, Michoacán, Guanajuato, Querétaro, Mexico City, Hidalgo, Colima, Tlaxcala, Puebla, Veracruz and Mexico City (Berlanga et al., 2020). This volcanic axis extends from the Pacific Ocean to the Gulf of Mexico (Peterson, 2020). Coccidia of the genus *Isospora* Schneider, 1881 are the most common in passerine birds (Duszynski et al., 1999; Berto et al., 2011). The 18S rRNA nuclear genes have been among the first genetic loci intended to obtain sequences for the molecular and phylogenetic characterization of coccidia (Barta et al., 1997; Carreño and Barta, 1999; Power et al., 2009; Yang et al., 2012; Chapman., 2013; Soriano-Vargas et al., 2017) and 18S RNA loci segment sequences continue to be widely used due to the large public database of reference sequences continue to be widely used due to the large public database of reference sequences. Many species of *Isospora* have been described from passerine birds worldwide (Schrenzel et al., 2005; Dolnik and Loonen, 2007; Berto et al., 2011; Yang et al, 2015). The aim of the present study is to document the findings of a new species of *Isospora* in this endemic bird

Material and methods

The sampling area was located on Santa María Hill (19° 41´ 10´´ N; 101° 10´ 52´´ W), on a longitudinal slope from 1,920 to 2,390 (m a.s.l.), Michoacán State (INEGI, 2025), where a habitat of the blue mulatto

was located. The bird was observed until defecation. Fresh fecal samples (N = 5) were collected at different times between dawn and moon. Fecal samples were collected with a disposable surgical microcollector (one per sample), avoiding the collection of urates and soil. They were then poured into plastic vials with a 2.5% potassium dichromate ($K_2Cr_2O_4$) solution 1:6 (v/v). Samples were spotted onto a thin layer (approximately 5 mm) of 2.5% $K_2Cr_2O_4$ solution in Petri dishes, incubated at 23–28°C, and monitored daily until 70% of the oocysts had sporulated. Oocysts were recovered by flotation in Sheather's sugar solution (G.E. 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements (n = 30), following the recommendations of Duszynski and Wilber (1997) and Berto (2014). A Karl Zeiss Axiostar Plus binocular microscope coupled to a RoSH FCCE digital camera was used. All measurements are in micrometers and are expressed as a range followed by the mean in parentheses

DNA extraction from faces and PCR sequencing

Total DNA from 250 mg of fecal sample was washed by centrifugation three times with sterile water and centrifuged for removal the potassium dichromate solution. Then, oocyst pellets were resuspended QIAampÒ Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the protocols of the manufacturer (Barta et al., 1997; Carreño and Barta, 1999; Power et al., 2009; Yang et al., 2012: Chapman et al., 2013). And then, by using cell disruption (SoniBeast™ BioEspec, United States), it is carried out in a cycle of 30 seconds, at an ambient temperature between 12–15° Celsius, MM 400, 30 Hz, avoiding cross contamination (Maekawa et al., 2024). The recommended beads for protozoan cell disruption are glass beads with a diameter of 0.75 to 1.0 mm (Jang-Ock et al., 2014; Grigorov et al., 2020)

Partial 18S rRNA gene fragments were amplified by performing nested PCR (Yang et al., 2012; Chapman et al., 2013; Soriano-Vargas et al., 2017). The DNA was extracted directly from oocysts and purified by using QIAampÒ Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the protocols of the manufacturer. The 18S ribosomal RNA (rRNA) gene was amplified from oocysts by using conditions and primers described by Yang et al. (2012): forward primer EIF1 5'GCTTGTCTCAAAGATTAAGCC-3' (previously described by Power et al., 2009; Chapman et al., 2013), reverse primer EIR3 5' ATGCATACTCAAAAGATTACC-3'. Products of the first PCR were used as template for a second amplification: forward primer EIF3 5'-CTATGGCTAATACATGCGCAATC- 3' and the reverse primer EIR3 to obtain a 1399–1407 bp fragment (Yang et al., 2012; Soriano-Vargas et al., 2017; Myeongsu et al., 2023). For PCR, GoTaq Flexi DNA Polymerase and dNTP Mix (PROMEGA, Wisconsin, USA) were used. Amplification was performed in 50 mL volumes containing 10 mL of GoTaq Flexi Buffer (5X), 4 mL MgCl2 solution (25 mM), 2 ml of PCR Nucleotide Mix (10 mM each dNTP), 2 mL of each primer, 5 mL of DNA template, 24.75 mL of PCR grade water and 0.25 mL of GoTaq Flexi DNA Polymerase (5 U/ mL). The PCR conditions consisted of an initial denaturation at 94 ºC for 5 minutes, followed by 30 cycles of denaturation at 94º C for 30 s, annealing at 57 ºC for 30 s, extension at 72° C for 2 minutes and a final extension at 72º C for 10 min. For EIF3 and EIR3 primers, the amplification reactions consisted of an initial denaturation at 94º C for 3 minutes, followed by 40 cycles of denaturation at 94º C for 30 s,

annealing at 60º C for 30 s, extension at 72º C for 1.5 minutes and a final extension at 72º C for 7 min. PCR products were analyzed by electrophoresis on a 1.0% (w/v) agarose gel in TBE buffer (1X) (PROMEGA, Wisconsin, USA), stained with ethidium bromide, and visualized in a UV transilluminator. The image was captured using a MiniBis Pro photo documentation system (DNR Bio-Imaging Systems, Israel). PCR products were purified using a QIAquick gel extraction kit (QUIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA products were quantified using an iCAP 6500 spectrophotometer (Thermo Fisher, Waltham, Massachusetts, USA). 18S rRNA sequencing was performed with oligonucleotides EIF1, EIF3, and EIR3, and sequencing reactions were carried out with the BigDye Teminator v. kit. 3.1 (Applied Biosystems, USA) on the 3730xl DNA Analyzer sequencer (Applied Biosystems, USA) of the Genomic Sequencing Laboratory for Biodiversity and Health, Institute of Biology of UNAM, Mexico

Analysis and editing of sequences

The sequences obtained (format. ab1) were assembled and edited in the Sequencher program version 4.8 (Genes Codes Corporation, USA), part of the ends that presented ambiguous or poor-quality bases were removed. With this same program, the sequences were exported to FASTA format (.fas) and uploaded to the “blastn” platform (NCBI, USA) to corroborate the amplification of the 18S region. Subsequently, the alignment was carried out with the PhyDE® program (Phylogenetic Data Editor) using the MUSCLE algorithm (Edgar, 2004) and the aligned sequences were exported to NEXUS format (.nex) for subsequent phylogenetic analysis (Rambaut et al., 2018)

Phylogenetic analysis

Phylogenetic trees were constructed of *Isospora* spp. using partial 18S aligned with additional isolates from GenBank. The phylogeny of the genus *Isospora* spp. It was inferred using the Maximum Likelihood and Bayesian Inference method. For Maximum Likelihood was performed using the bioinformatics program PhyML version.3.0 (Guindon et al., 2010, RRID: SCR_014629) using the Smart Model Selection (SMS) software (Lefort et al., 2017) on the ATGC Montpellier Bioinformatics platform (http://www.atgc-montpellier.fr/, RRID:SCR_002917). The nucleotide substitution (evolution) model was TN93 + R according to the Akaike information criterion (-lnL= -12131.12252, AIC = 24576, 24504. Probability-based branch support measures (approximate likelihood ratio test [aLRT] and Shimodaira-Hasegawa [SH]-aLRT) were required to have greater precision of the tree obtained, which was evaluated with a Bootstrap after every 1,000 permutations.

For the Bayesian Inference tree, 18S rRNA gene sequences were aligned in Clustal X version 2.0 (Larkin et al., 2007, RRID:SCR_017055). Default parameters were used for the opening and extension of spaces, the inference was carried out in the BEAST2 program version 2.5 (Bouckaert et al., 2019, RRID:SCR_017307) the previous estimated parameters were through the GTR nucleotide substitution model and the Yule model. The horizontal distance is proportional to the hypothetical Bayesian

evolutionary change. The support (probability) of the inference node (IB) is indicated for all nodes greater than **0.5**. Three independent Markov chains were run for 10,000,000 million generations, sampling every 10,000 of them. The Tracer bioinformatics model version 1.7.2 (Rambaut et al., 2018, RRID:SCR_019121) was used to verify trace convergence and effective sample size values. 10% of the sampled trees were discarded as burn-in using the LogCombiner algorithm (Bouckaert et al., 2019) and through the TreeAnnotator program (Bouckaert et al., 2019) to collapse the tree distribution and Bayesian posterior probability values resulting in a maximum clade credibility tree

Morphological analysis

Sporulated coccidian oöcysts were observed using a Karl Zeiss Axiostar plus microscope (176045 Germany) using 10X and 40X objective lenses, and measurements and captures were performed using a 100X oil immersion objective and an ocular micrometer. All measurements are presented in micrometers, with means in parentheses after ranges. Line drawings were edited using two CorelDRAW® software applications (Corel Draw Graphics Suite, version 2020, Corel Corporation, Canada): Corel DRAW and Corel PHOTO-PAINT (Yang et al., 2021)

Results

The fecal 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), this material is described below

Family Eimeriidae Michin, 1903

Genus Isospora Schneider, 1881

Isospora melanotisi n. sp

Type-host: *Melanotis melanotisi* (Aves: Passeriformes: Mimidae), Blue Mockingbird

Type-locality

Área Natural Protegida La Loma de Santa María (19° 41´ 10´´ N; 101°10´52´´ W), Morelia, State of Michoacán, México

Type-material

Oöcysts in dichromate solution, phototypes and line drawings of sporulated oöcysts are deposited and available in (www.AprotectA.org) in the Repository Parasitology area of the Michoacán veterinary faculty, Mexico, the repository number is CGL-04/2026

Prevalence

Oöcysts of this species were found in 1/1 (100%) on the fresh faecal samples examined

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), the details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for **Isospora melanotisi** is urn:lsid:zoobank.org:pub:27165BF1-2D7E-4090-A112-3AFC9F63CC61

Etymology

The name is derived from the species name of type-host

Description (Figs. 1 and 2)

Sporulated oöcyst

Oöcysts (n = 30) are ovoid, 25–29 X 21–25 (26.9 x 23.1); Length/Width (L/W) ratio 1.0–1.1 (1.04) Wall bi-layered 0.6–1.1 (0.8) thick, outer layer clear and dark interior. One or two polar granules, micropyle and oöcyst residuum absents

Sporocyst and sporozoites

Sporocyst (n = 20) are avoid, to drop-shaped 14–22 x 10–13 (16.6 x 11.0); L/W ratio 1.4–1.7 (1.6) Stieda body present, Knob-like shape 8.1 high 2.0 wide; sub Stieda body present rounded 2.3 high, 3.7 wide; para Stieda body absent; sporocyst residuum present and diffuse mass of granules Sporozoites 4, vermiform shape 10.1–11.4 (11.0) 4.3–4.7 (4.4)

Remarks

Boughton and Volk (1938), reported six *Isospora* sp. of Passerine birds of the Mimidae family. The following species respectively Host; *Dumetella carolinensis* (Gray catbird), *Melanotis melanotisi* (Blue mockingbird), *Mimus gilvus* (Tropical mockingbird), *Mimus polyglottos* (Northern mockingbird), *Toxostoma rufum* (Brown thrasher) (Berto et al., 2011)

Figure 1 Photomicrograph of sporulate oöcyst of *Isospora melanotisi* n. sp. Ovoid sporocyst (DOW) doble-layered outer wall clear and dark interior, Stieda body (SB) rounded and clear visible sub-Stieda body (SSB) with the presence of one or two polar granule (PG) and a diffuse sporocyst residuum (SR). *Scale-bar* 10 µm

Table 1
Comparative morphology of *Isospora* spp. from species of the Mimidae family

Species	***Isospora melanotisi* n. sp**	***Isospora toxostomai* n. sp.**	***Isospora mimusi***
Host	*Melanotis caerulescens* (Swainson)	*Toxostoma curvirostre* (Swainson)	*Mimus gilvus* (Vieillot)
Locality	Morelia, State Michoacán, Mexico	Toluca Valley, México	Rio de Janeiro, Brazil.
Reference	Present study	Salgado et al. (2019)	Coelho et al. (2011)
Oöcyst			
Shape	Ovoid	Subspherical	Subspherical
Wall	Bi-layered	Bi-layered	Bi-layered
Length (L)	25–29 (26.9)	22–25 (23.4)	19–23 (20.6)
Width (W)	21–25 (23.1)	21–24 (22.3)	18–22 (19.6)
L/W ratio	1.0–1.1 (1.1)	1.0–1.1 (1.1)	1.0–1.1 (1.1)
Polar granules	Present one or two	Present, many fibrils	Absent
Oöcyst residuum	Absent	Absent	Absent
Sporocyst			
Shape	Ovoid	Ovoid	Ovoid
Length (L)	14–22 (16.6)	15–17 (15.8)	11–16 (13.4)
Width (W)	10–13 (11.0)	10–11 (10.5)	9–11 (9.8)
L/W ratio	1.4–1.7 (1.6)	1.3–1.6 (1.5)	
Stieda body	Knob-like	Knob-like	Flattened
Sub-Stieda body	Rounded	Trapezoidal; compartmentalized	Prominent, homogeneous
Residuum	Diffuse	Diffuse	Diffuse
Sporozoite			
Shape	Vermiform	Vermiform	Vermiform
Length (L)	10.1–11.4 (11.0)	13.5–14.7 (14.0)	*c.*13.0
Width (W)	4.3–4.7 (4.4)	3.0–3.3 (3.1)	*c.*4.0

Measurements in micrometers (μm) and abbreviations follow Wilbert et al., 1998

Phylogenetic analysis

The PCR products were aligned with 36 other *Isospora* sp. sequences identified with the 18S rRNA gene in GenBank of passerine birds, for the construction of two phylogenetic trees; by Maximum Likelihood (MV) (Fig. 3). Selection of reference sequences was based on NCBI BLAST similarities (one sequence per species). *Toxoplasma gondii* was used to cover all *Isospora* sp. sequences, plus one *Cryptosporidium andersoni* sequence (HQ259590.1) as an outgroup. MV analysis revealed that *I. caeruleum* n. sp. indicates its closeness with 76% with *I.* cf. *erithaci Luscina Suecica* (MK559091.1), indicating that they share a common ancestor, but on a different branch of the cladogram (Yang et al., 2016). Regarding the Inference Bayesian (IB) tree (Fig. 4), the common ancestor is *Isospora* sp. isolate Sk01 *Sturnus vulgaris* (MN639700.1) passerine bird native to Europe and Africa Passeriformes of the family Sturnidae forming a subsequent support clade of 0.8581 with *Isospora* sp. identified in the second cladogram as *Melanotis caerulescens* 14C

Discussion

In Michoacán, México, 561 species of birds are recorded, belonging to 24 orders and 72 families. Regarding the birds of the order Passeriformes, 256 species are registered, within 46 families (Villaseñor and Villaseñor, 2005), in Protected Natural Area La Loma de Santa María, 70% of these species have been observed and documented (iNaturalist, 2023). To date, not helminth or protist parasites have been described in *Melanotis caerulescens*. Of the approximately 35 species of the Mimidae family in the world (more than 75% in America) (Beletski, 2025), only thirteen studies have currently been carried out and only three on their health status. Identifications of new species of coccidia should be based first on comparison morphology among recorded coccidian species in the same family as the host. The description of coccidia species is fundamentally based on the comparative morphology of the sporulated oocysts (Duszynski and Wilber 1997). In this case the sporulated oocyst is larger and ovoid in shape compared to the findings of *Isospora toxostomai* n. sp (Salgado, 2019) and *Isospora mimusi* (Coelho et al., 2011) (Table 1). The result of the MV analysis shows that in the *Isospora* sp. cladogram. from *Melanotis caerulescens* 14C, is close to *I. cf. erithaci Luscina Suecica* (MK559091.1), a passerine of the family Muscicapidae, a bird belonging to the Old World with a 76% probability, which demonstrates its diversification within the range of the phylogenetic method used, demonstrating that it belongs to a different phylogenetic branch. Regarding the IB analysis, at posterior probability (0.8581), it ensures a high clustering of genetic divergence compared to its closest ancestors, it belongs to a separate monophyletic clade which confirms that it is a separate biological entity

Following the host family specificity criterion, widely accepted for passerine coccidia (Duszynski and Wilber, 1997; Berto et al., 2011; Berto et al., 2014). Complete and reliable studies on the taxonomy of coccidia in wild birds have demonstrated specificity in which unfortunately there is not much research on the matter, at least in the country. In the morphological observations of the sporulated oocyst, it is observed that they differed in measurements. Therefore, they must be complemented with molecular

studies for greater certainty in the characterization of a new species (Bouckaert et al., 2019; Yang et al., 2021)

Declarations

Acknowledgements Parasitology Area of the Auxiliary Diagnostic Services Unit, Faculty of Veterinary Medicine and Zootechnics, Universidad Michoacana de San Nicolás de Hidalgo, State of Michoacán, Mexico

Author Contributions This study was performed by the authors, who performed laboratory procedures for maintenance, recovery, measurements, photomicrographs, and oöcyst isolation. CGL drew the coccidian oöcyst. The Manuscript was written by the three authors

Funding This study was funded by its authors

Data availability Phototypes, line drawing, and oöcyst in 70% ethanol are deposited and available file:///C:/Users/Clau%20Guz/Downloads/pagina_web_laboratorio_de_parasitologia_umsnh.html repository of the Parasitology Area of the Michoacan Veterinary Faculty. México, under repository number CGL-02/2025, along with the photographs of type-host specimen (symbiotype)

Conflict of interest the authors declare that they have no conflict of interest

Ethical approval All applicable institutional, national and international guidelines for care and use of animals were followed

References

1. Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner S, Profous-Juchelka H. Phylogenetic Relationships among Eight *Eimeria* Species Infecting Domestic Fowl Inferred Using Complete Small Subunit Ribosomal DNA Sequences. The Journal of Parasitology. 1997; 83: 262–271.
2. Belesky L. (2025). *Birds of the World.* Cornell Lab of Ornithology. Ed. Macaulay. Cornell University Ithaca, New York, USA. 119123.
3. Berlanga H, Gómez de silva H, Vargas-Canales VM, Rodríguez-Contreras V, Sánchez-González LA, Ortega-Álvarez R, Calderón-Parra R. (2020): *Aves de México*: Lista actualizada de especies y nombres comunes. CONABIO, México. 1118.
4. Berto BP, Flausino W, McIntosh D, Teixeira-Filho WL, Lopes CWG. Coccidia of New World passerine birds: a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa Eimeriidae). Systematic Parasitology. 2011; 80: 159–204.
5. Berto BP, McIntosh D, Gomes-Lopes CW. Studies on coccidian oocyst (Apicomplexa: Eucoccidiorida). Brazilian Journal of Veterinary Parasitology. 2014; 23: 1–15.

6. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Drummond AJ. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Computational Biology. 2019; 15: 1–128.
7. Boughton DC. Avian Hosts of Genus *Isospora* (Coccidiida). Zoology Department. University of Georgia. USA. 1939; 38: 149–153.
8. Carreño RA and Barta JR. An Eimeriid Origin of Isosporoid Coccidia with Stieda Bodies as Show by Phylogenetic Analysis of Small Subunit Ribosomal RNA Gene Sequences. The Journal of Parasitology. 1999; 85: 77–83.
9. Chapman HD, Barta JR, Blake D, Gruber A, Jenkins M, Smith CN, Suo X, Tomey MF. Selective review of advances in coccidiosis research. Advances in Parasitology. 2013; 83: 93–171.
10. Coelho CD, Berto BP, Neves DM, de Oliveira VM, Flausino W, Wilson. Lopes CWG. Two new *Isospora* species from the saffron finch, *Sicalis flaveolata* in Brazil. Acta Parasitologica. 2011; 56: 239–244.
11. Dolnik OV and Loonen MJE. *Isospora plectrophenaxia* n. sp (Apicomplexa: Eimeriidae), a new coccidian parasite found in Snow Bunting (*Plectrophenax nivalis*) nestlings on Spitsbergen. Parasitology Research. 2007; 101: 16171619.
12. Duszynski DW, Couch L, Upton SJ. The coccidia of the world. 1999. Accessed April 2020. http://www.k-state. edu/parasitology/worldcoccidia/index.html
13. Duszynski DW and Wilber PG. A Guideline for Preparation of Species Descriptions in the Eimeriidae. Journal of Parasitology. 1997; 83: 333–336.
14. Edgar RC. MUSCLE. Multiple Sequence Alignment with high Accuracy and high Throughput. Nucleic Acids Research, 2004; 32: 1792–1797.
15. Grigorov E, Kirov B, Marinov MB, Galabov V. Review of Microfluidic Methods for Cellular Lysis. Micromachines (Basel). 2021. 28; 12 (5): 498499.
16. Guindon S, Dufayard J, Lefort V, Anisimova M, Hordijk W, Gascuel O. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology. 2010; 59; 307–321.
17. iNaturalist. Guía de la Zona sujeta a restauración y protección ambiental Loma de Santa María y depresiones aledañas, Morelia, Michoacán. Agosto 2023. https://www.inaturalist.org/guides/4589?taxon=7251
18. International Code of Zoological Nomenclature (ICZN). 4th ed. 2012.
19. Instituto Nacional de Estadística y Geografía (INEGI). (2025). La Loma de Santa María. Morelia, Michoacán, México. Recuperado de https://www.morelia.gob.mx/loma-de-santa-maria/
20. Jang-Ock C, Abul FS, Muhammad T, Chae WL, Bumseok K. Effects of glass bead size, vortexing speed and duration on *Eimeria acervulina* oocyst excystation. Experimental Parasitology. 2014; 138: 18–24.
21. Knight A, Ewen GJ, Brekke P, Santure WA. The evolutionary biology, ecology and epidemiology of coccidia of passerine birds. Advances in Parasitology. 2018; 99: 35–60.

22. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R. Clustal W and Clustal X version 2.0. Bioinformatics, 2007; 23: 2947–2948.
23. Lefort V, Longueville J and Gascuel O. SMS: Smart Model Selection in PhyML. Molecular Biology and Evolution. 2017; 34: 2422–2424.
24. Maekawa AS, Santos LS, Velho PE and Drummond MR. Use of a synthetic oligonucleotide to detect false positives caused by cross-contamination in nested PCR. Journal of Microbiological Methods. 2024; 226: 107040.
25. Myeongsu K, Phyo WW, Yoonhee K, Seulgi G, Haerin R, Jae-Ik H. Prevalence and Phylogenetic Analysis of Coccidian Parasites from Wild Animals with Diarrhea in Jeonbuk Province, Korea. Journal of Veterinary Clinics. 2023; 40: 189–196.
26. Peterson R. (2020). *Field guide to Birds of North America*. Second Ed. HarperCollins editors. 210230.
27. Peterson, RT, y Edward L. (2008). *Aves de México*. Guía de campo. Editorial Diana, México. ISSN 006-1719. 4971.
28. Power ML, Ritcher C, Emery S, Hufschmid J, Gillings MR. *Eimeria trichosuri*: phylogenetic position of a marsupial coccidium, based on 18S rDNA sequences. Experimental Parasitology. 2009; 122: 165–168.
29. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology. 2018; 67: 901–904.
30. Salgado-Miranda C, Medina JP, Sánchez-Jasso JM, García-Albarrán M, Soriano-Vargas E. *Isospora toxostomai* n. sp. (Apicomplexa: Eimeriidae) from the curved-billed thrasher Toxostoma curvirostre (Swainson) (Passeriformes: Mimidae) at the Central highlands of Mexico. Systematic of Parasitology. 2019; 96; 789793.
31. Schrenzel MD, Maalouf GA, Gaffney PM, Tokarz D, Keener LL, McClure D, Griffey S, McAloose D, Rideout BA. Molecular Characterization of *Isosporoid* Coccidia (*Isospora* and *Atoplasma*) in Passerine Birds. Journal of Parasitology. 2005; 91: 635–647.
32. Secretaria de Medio Ambiente y Sustentabilidad. Medio ambiente y sustentabilidad. Loma de Santa María. Zona sujeta a preservación ecológica. Fecha de Decreto: 31 de diciembre del 2009. 170.5 has. https://medioambiente.morelia.gob.mx/loma-de-santa-maria/
33. Soriano-Vargas E, Medina JP, Salgado-Miranda C, García-Conejo M, Galindo-Sánchez KP, Krzysztof JM, Berto BP, Gomes LCW. *Eimeria pileata* n. sp. (apicomplexa: Eimeriidae) from rufous-capped brush finch *Atlapetes pileatus* Wagler (Passeriformes: Emberizidae) in México. Systematic Parasitology. 2015; 92: 261–265.
34. Soriano-Vargas E, Salgado-Miranda C, Zepeda-Velázquez AP, Medina JP, Krzysztof JM, González-Gómez M, Flores-Valle IT, Berto BP, Gomes LCW. *Eimeria atlapetesi* nom. Nov., a replacement name for *Eimeria pileate* (Apicomplexa: Eimeriidae), preoccupied by *Eimeria pileata* Straneva and Kelley, 1979 (Apicomplexa: Eimeriidae), with observations on histopathology and phylogenetic analysis. Zootaxa. 2017; 140–150.

35. Tenter MA, Barta RJ, Beveridge I, Duszynski WD, Mehlhorn H, Morrison AD, Thompson RCA, Conrad AP. The conceptual basis for a new classification of coccidia. International Journal for Parasitology. 2002; 32: 595–616.
36. Wilber PG, Duszynski DW, Upon SJ, Seville RS, Corlis JO. A revision of the taxonomy and nomenclature of the *Eimeria* spp. (Apicomplexa: Eimeriidae) from rodents in the Tribe Marmotini (Sciuridae). Systematic Parasitology. 1998; 39: 113135.
37. Villaseñor GLE, Villaseñor GJF. Especies y Subespecies de Aves en Michoacán, México. Ornitología. Universidad Michoacana de San Nicolás de Hidalgo. 2005; 23–49.
38. Yang R, Brice B, Berto BP, Ryan U. Morphological and molecular description of a new species of *Isospora* (Apicomplexa) from a New Holland honeyeater (*Phylidonyris novaehollandiae*). Parasitology International. 2021; 83: 102348.
39. Yang R, Brice B, Jian F, Ryan U. Morphological and molecular characterization of *Isospora manorinae* n. sp. in a yellow-throated miner (*Manorina flavigula wayensis* (Gould, 1840). Experimental Parasitology. 2016. 163; 1623.
40. Yang R, Brice B, Berto BP, Zahedi A. Morphological and molecular characterization of a new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the western wattlebird *Anthochaera lunulata* Gould in Western Australia. Current Research in Parasitology &Borne Diseases. 2021. 1: 18.
41. Yang R, Fenwick S, potter A, Elliot A, Power M, Beveridge I, Ryan U. Molecular characterization of *Eimeria* species in macropods. Experimental Parasitology. 2012; 132: 216–221.
42. Yang R, Brice B, Habsi AK, Elliot A, Ryan U. *Isospora streperae* n. sp. (Apicomplexa: Eimeriidae) from a grey currawong (*Strepera versicolour plumbea*) (Passeriformes: Artamidae) in Western Australia. Experimental Parasitology. 2015; 1–17.

Figures



Figure 1

Photomicrograph of sporulate oöcyst of *Isospora melanotisi* n. sp. Ovoid sporocyst (DOW) doble-layered outer wall clear and dark interior, Stieda body (SB) rounded and clear visible sub-Stieda body (SSB) with the presence of one or two polar granule (PG) and a diffuse sporocyst residuum (SR). *Scale-bar* 10 µm

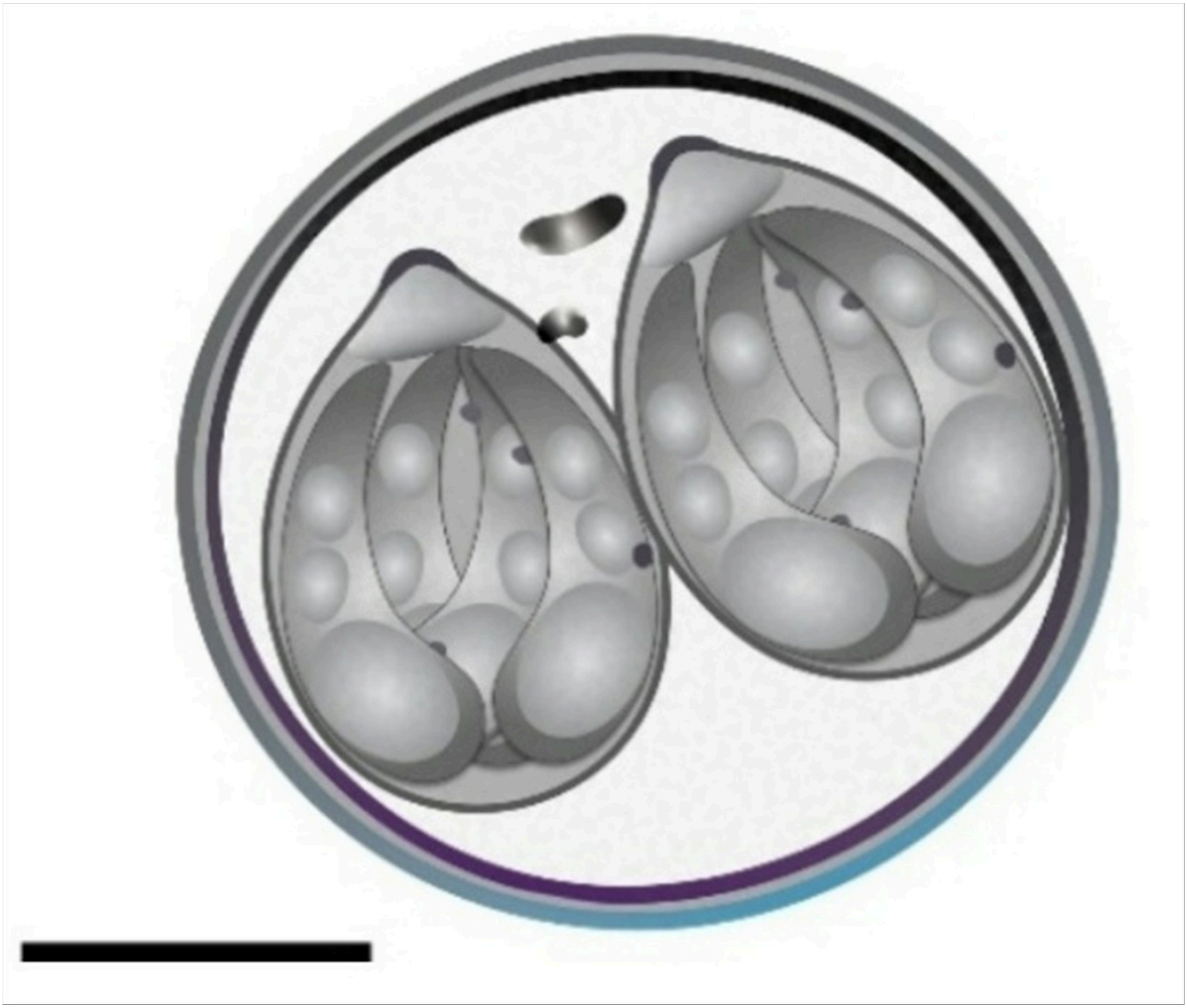


Figure 2

Line drawing of a sporulated oöcyst of *Isospora caeruleum* **n. sp.** from *Melanotis caerulescens. Scale-bar* 10 µm

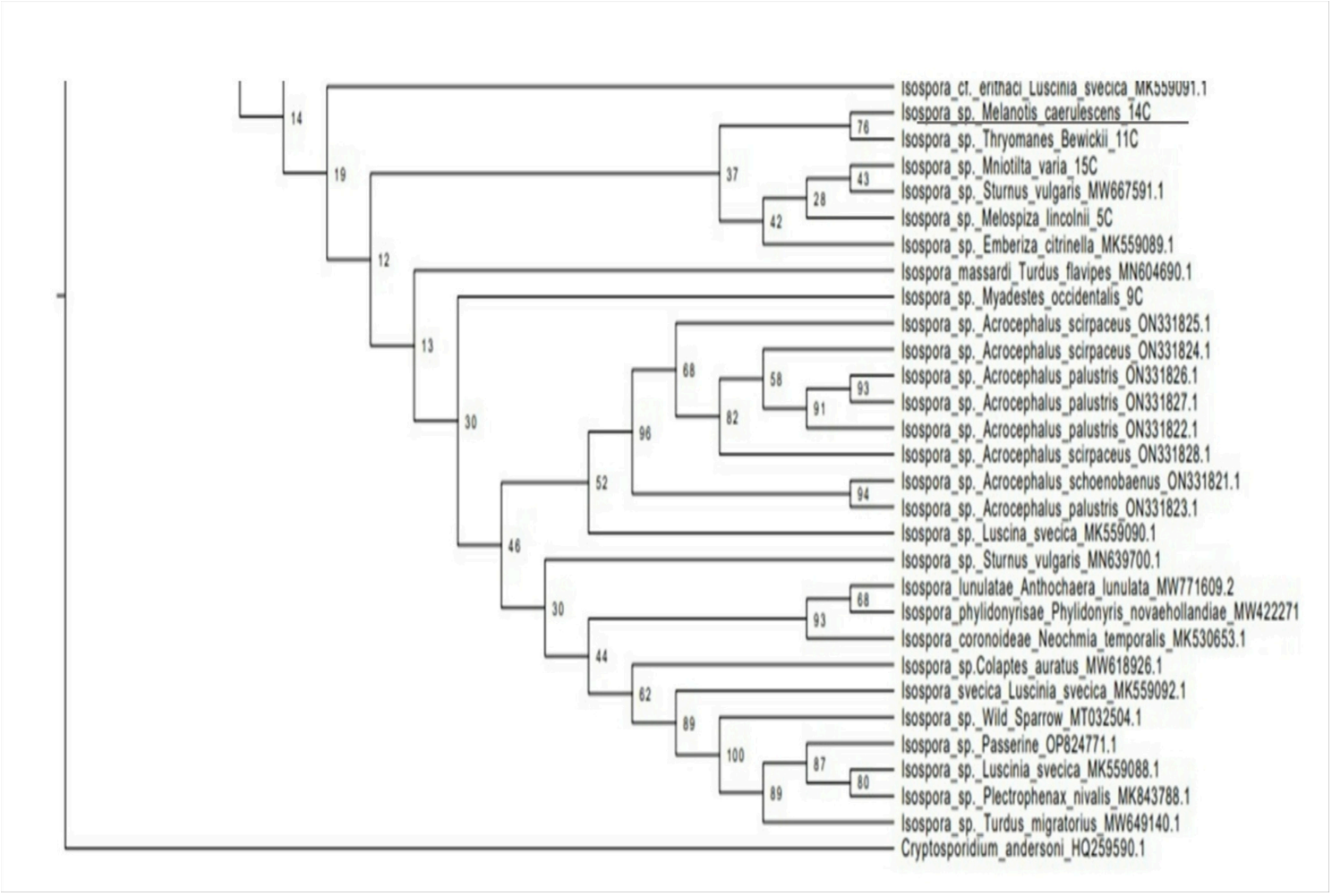


Figure 3

Phylogenetic tree of *Isospora melanotisi* sequences. through the 18S rRNA gene, reconstructed by Maximum Likelihood (ML), using F81 as an evolutionary model in the IQTree2 program. The support value of the branches corresponds to 1,000 Bootstrap replicates indicated at the nodes, the measures based on approximate probability. Percentage support (>70%)

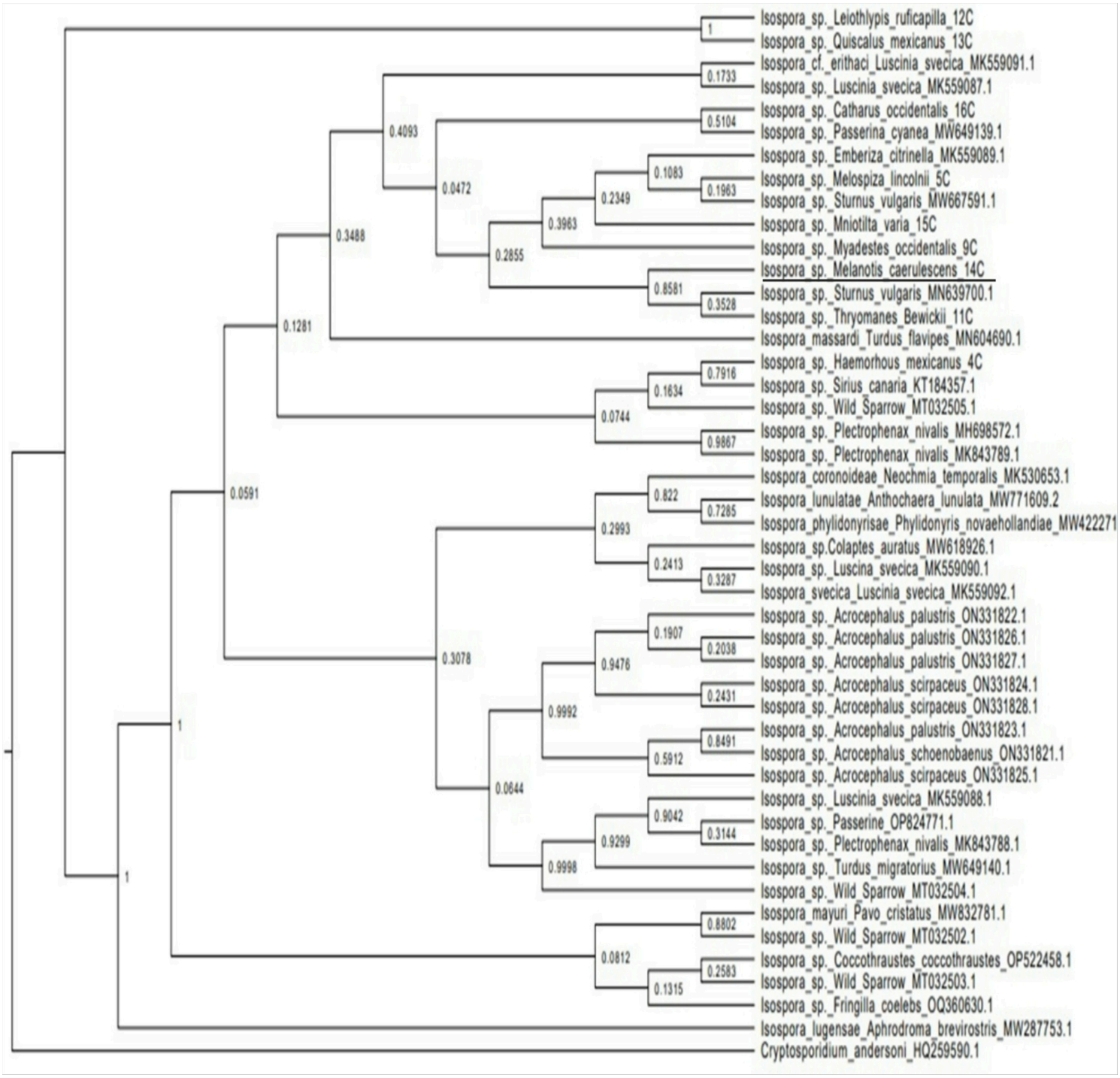


Figure 4

The present Bayesian Inference tree has a node support (probability) value that is indicated for all to be greater than 0.5. Three independent Markov chains were run for 10 million generations, sampling every 10,000 of them