Acta Protozool. (2010) 49: 121–124
http://www.eko.uj.edu.pl/ap

ACTA
PROTOZOOLOGICA

Isospora dendrocinclae n. sp. (Apicomplexa: Eimeriidae) from the White-chinned Woodcreeper (*Dendrocincla merula*) from South America

Thomas E. McQUISTION[1], Samuel GALEWSKY[1], Angelo P. CAPPARELLA[2] and Fredrick REBLING[3]

[1]Department of Biology, Millikin University, Decatur, Illinois; [2]Department of Biological Sciences, Illinois State University, Normal, Illinois; [3]George Washington University, Washington, D.C. USA

Summary. A new species of *Isospora* is described from the fecal contents of the white-chinned woodcreeper, *Dendrocincla merula merula* from Guyana and *Dendrocincla merula barletti* from Peru. Sporulated oocysts are subspherical to ovoid, 19.2 × 16.5 (15–23 × 14.5–19) µm, with a smooth, colorless, bilayered wall. The average shape index is 1.2. No micropyle or oocyst residuum are present, but the oocysts contain one polar granule. Sporocysts are ovoid, 12.9 × 8.3 (12–14 × 7–10) µm, average shape index of 1.7 with a smooth, single layered wall and composed of a small, knoblike Stieda body and a slightly larger, bubble shaped substieda body. The two sporocysts each contain a compact residuum composed of coarse, non-uniform granules and four randomly arranged, vermiform sporozoites each with a terminal refractile body and a centrally located nucleus. DNA sequences representing ITS-1 and ITS-4 regions of the 5.8S rDNA gene from the two isolates were amplified and compared. In addition to the two isolates showing similar morphological characteristics, they also had identical nucleotide sequences for the ITS-1 and ITS-4 regions of the 5.8S ribosomal gene.

Key words: *Isospora dendrocinclae*, Passeriformes, Dendrocolaptidae, phylogenetics, ribosomal RNA, molecular systematics.

INTRODUCTION

The white-chinned woodcreeper's (*Dendrocincla merula*) range mainly covers lowland Amazonia and includes the eastern regions of Colombia, Ecuador, and Peru; northern Bolivia; Amazonian Brazil; the Guianas; and southern Venezuela (Ridgely and Tudor 1994). They forage largely in pairs or small family groups for invertebrates, and are often associated with army ant swarms (del Hoyo *et al.* 2003). Like other undergrowth tropical birds that inhabit primarily terra firme forest, they are thought to be sedentary (Capparella 1988, Brown *et al.* 2004). There are no reports of coccidian parasites from *D. merula*. This paper describes a new isosporan parasite from two *Dendrocincla merula* populations collected in Guyana and Peru at opposite ends of *D. merula's* range.

In addition to morphological comparison between the two isosporan isolates from *D. merula*, genetic analysis of the ribosomal Internal Transcribed Spacer (ITS1 and ITS4) were isolated and amplified to compare genetic similarity (Barta 2001).

Address for correspondence: Thomas E. McQuistion, Department of Biology; Millikin University, Decatur, Illinois 62522, U.S.A. (217) 424-6236; Fax (217) 424-3958; E-mail: tmcquistion@millikin.edu

MATERIALS AND METHODS

Oocyst collection

Fecal samples were collected from six white-chinned woodcreepers during a bird collecting expedition in June 1996 from Guyana and Peru. Upon collection, the fecal samples were placed in 2.5% (w/v) $K_2Cr_2O_7$ and maintained at cool temperatures in the field. Upon arrival at the laboratory, the samples were processed and examined as described by McQuistion and Wilson (1989). A Nikon Labophot® microscope with an optical micrometer and a dark box with a HFX-DX photomicrographic attachment were used to measure and photograph the oocysts. All measurements are given in micrometers with the size ranges in parenthesis following the means. Oocysts were two months old when originally examined, measured, and photographed.

DNA isolation, amplification, and sequencing

A Qlamp® DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) was used to extract the DNA from the oocysts. The manufacturer's DNA isolation protocol was followed with the following modifications. The InhibitEX® (inhibitor-adsorption tablets) were not used. Instead, oocysts from the Guyana and Peru isolates were cleaned with standard bleach and washed three times in 0.9% saline solution. Before the first incubation step (as indicated in the protocol) 3 mm sterile glass beads (Kimble Glass, Vineland, New Jersey) were added to the microcentrifuge tube containing the oocysts and the mixture was vortexed for 5 minutes to disrupt the oocysts for extraction of the DNA.

PCR amplification was carried out using a Perkin Elmer® Gene Amplication (PCR) System. The PCR mixture was 1 µl of DNA, 2 µl ITS-1, 2 µl ITS-4 and 5 µl Master Mix® (Promega Biotech, USA). The mixture was placed in a PCR tube and amplified by the technique described by Barta *et al.* (1998). The sequence chosen for DNA analysis was the Internal Transcribed Spacer (ITS). The primers used for PCR were ITS-1 (5'>3') TCCGTAGGTGAACCTGCGG and ITS-4 (5'>3') TCCTCCGCTTATTGATATGC (Barta *et al.* 1998). The primers were obtained from Sigma Genosys® (USA) and used at a concentration of 30 ng/µl. The desired product was extracted from agarose gel using a QlAquick® Gel Extraction Kit (QlAGen Sciences, Maryland, USA) and the manufacturer's protocol was followed without modification.

DNA sequencing was conducted using the Big Dye® PCR Cycle Sequencing System and an Automated Fluorescents DNA Sequencer. FinchTV version 1.4 (www.Geospiza.com) was used to view the DNA sequence. The Basic Local Alignment Search Tool (BLAST)(http:www.ncbi.nlm.nih.gov/blast) was used to compare obtained sequence fragments with known parasite lineages.

RESULTS

Isospora dendrocinclae n. sp. (Figs 1, 2)

Description of oocysts from type host: Sporulated oocysts are subspherical to ovoid, 19.2 × 16.5 (15–23, SD = 1.5 × 14.5–19, SD = 0.90 (N = 30) with a smooth, bilayered wall; the inner wall is thinner and darker than the outer wall. The shape index (length/width) is 1.2 (1.1–1.3, SD = 0.07). Micropyle and oocyst residuum are absent but one, subspherical, rough polar granule is present. Sporocysts are ovoid, 12.9 × 8.3 (12–14, SD = 0.80 × 7–10, SD = 0.61); shape index is 1.7 (1.6–1.8, SD = 0.09). The Stieda body is small and knoblike and the substieda body is slightly larger than the Stieda body and subglobular. Each sporocyst contain a subspherical residuum composed of coarse, non-uniform granules and four vermiform sporozoites randomly arranged in the sporocyst with a terminal, posterior refractile body and a centrally located nucleus.

Type host: *Dendrocincla merula merula* (Lichtenstein, 1820) white-chinned woodcreeper (Passeriformes: Dendrocolaptidae).

Type specimens: A phototype series and buffered formalin-preserved sporulated oocysts of *Isospora dendrocinclae* sp. n. are deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska 68588, accession no. HWML 63509 (syntypes) and HWML 49088 (phototypes).

Type locality: Guyana, Iwokrama Reserve, Kabocalli Landing, West bank of Essequibo River, ca. 45 river miles SE Kurupukari. 4°17′′N, 58°31′′W.

Location of symbiotype host specimen: Academy of Natural Sciences, Philadelphia, Museum no. ANSP 188562.

Other host: *Dendrocincla merula bartletti* Chubb, 1919.

Other host locality: Peru, Loreto Departamento, 79 km WNW of Contamana, ca. 400m elevation, 7°8′S, 75°41′′W.

Prevalence: 1/1 (100%) from Guyana location, 5/13 (38%) from Peru location.

Sporulation time: Unknown; oocysts were partially sporulated when received at the laboratory and became fully sporulated after exposed to air for several days prior to examination.

Site of infection: Unknown, oocysts found in feces.

Etymology: The specific epithet, *dendrocinclae,* is the Latin genitive form of the host genus name.

DNA analysis

DNA amplification of the Guyana and Peru isolates showed clear bands around 400 bp. The 5.8S rDNA sequence alignment indicated an identical sequence (100%) between the two isolates. The nucleotide se-

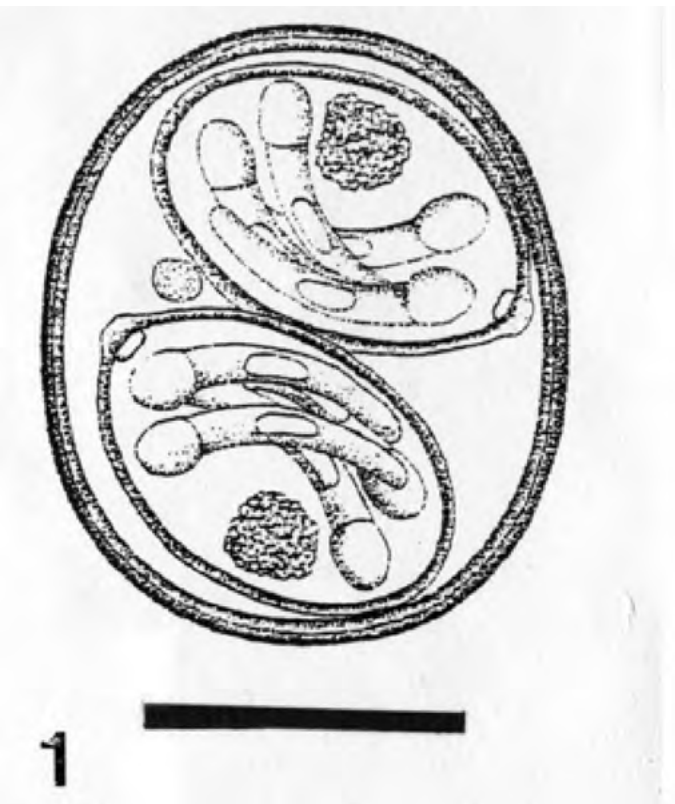

Fig. 1. Composite line drawing of sporulated oocyst *of Isospora dendrocinclae* n. sp. from the white-chinned woodcreeper, *Dendrocincla merula*. Scale bar: 10 µm.

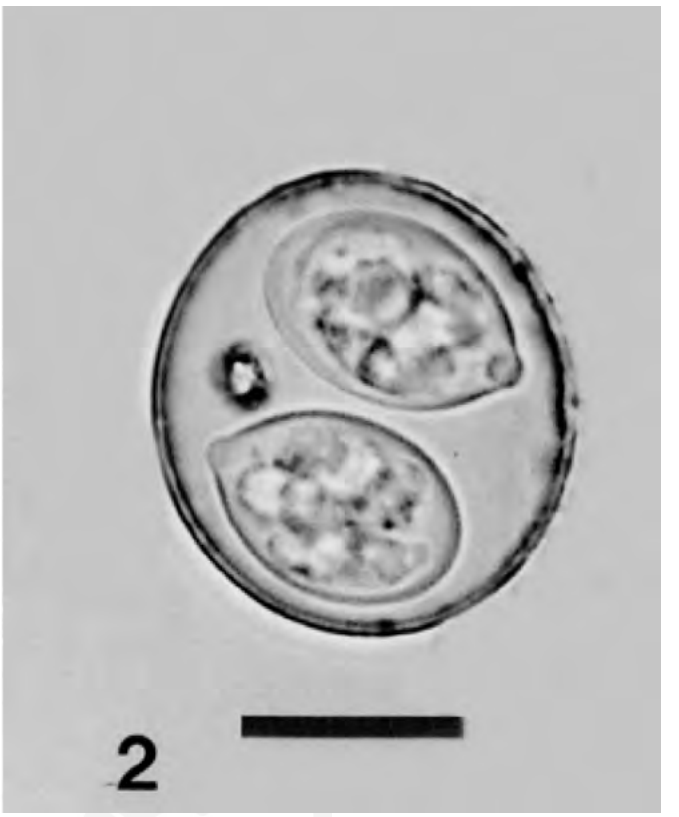

Fig. 2. Bright field photomicrograph of sporulated oocyst of *Isospora dendrocinclae* n. sp. from the white-chinned woodcreeper, *Dendrocincla merula*. Scale bar: 10 µm.

quences for the isolates are deposited in GenBank. The Guyana isolate accession number is GU952797 and the Peru isolate accession number is GU952798.

DISCUSSION

The white-chinned woodcreeper has seven named subspecies (Peters 1951). Two of the subspecies (nominate *merula* and *obidensis*) are distributed north of the Amazon River and east of the Rio Negro River and may constitute the single subspecies, *D. m. merula*, based on size, iris color and vocalizations (del Hoyo *et al.* 2003). *D. m. bartletti* (the Peru subspecies sampled) is confined to southern Venezuela on the upper Orinoco River south to northern Peru. Therefore*, Isospora dendrocinclae* was found in two subspecies of woodcreeper, one containing nominate *merula* and one containing nominate *bartletti.* No morphological differences were apparent between sporulated oocysts from these two coccidian isolates and the DNA sequences of ITS-1 and ITS-4 regions of their 5.8S ribosomal gene were identical.

Five isosporoid coccidia have been previously described and compared from three woodcreeper species in the family Dendrocolapidae (McQuistion and Capparella 1997). All three avian host species are sympatric with *Dendrocincla merula* in part of its range. *Isospora magna* and *I. concentrica* were described from the barred woodcreeper *Dendrocolaptes certhia* (McQuistion and Capparella 1995). Their oocysts and sporocysts are much larger than those of *I. dendrocinclae*. The oocysts of *I. magna* average 29.7 × 24.9 µm and *I. concentrica* average 26.6 × 22.7 µm compared to 19.2 × 16.5 µm for *I. dendrocinclae*. The sporocysts of *I. magna* average 15.8 × 12.6 µm and those of *I. concentrica* average 17.2 × 11 µm compared to 12.9 × 8.3 µm for

sporocysts of *I. dendrocinclae.* In addition, the shape indexes of the sporocysts are different for the three coccidian species; 1.3 for *I. magna*, 1.6 for *I. concentrica*, and 1.7 for *I. dendrocinclae.* Other differences between the three coccidian species include shape of the Stieda and substieda body on the sporocysts and the number of refractile bodies and unique characteristics of the sporozoites. The Stieda bodies of *I. magna* are broad, dome-like with an inconspicuous substieda body located directly below the Stieda body but slightly to the left or right of the vertical axis with a wavy lower surface. The Stieda bodies of *I. concentrica* are square or block-shaped with a bubble-shaped substieda body. The Stieda bodies of *I. dendrocinclae* are knoblike with a subglobular substieda body. Additionally, there are significant differences between the sporozoites of the three isosporan species. The sporozoites of *I. magna* are sausage-shaped and contain refractile bodies at each end of the sporozoite while *I. concentrica* have striations marking the anterior half of the sporozoite. In comparison, the sporozoites of I. dendrocinclae are vermiform with one terminal refractile body and no striations.

Isospora ubique was described from the wedge-billed woodcreeper, *Glyphorynchus spirurus* (McQuistion and Capparella 1997*). I. ubique* oocysts are larger (23.4 × 21.8 µm) than *I. dendrocinclae* (19.2 × 16.5) and subspherical (shape index is 1.07 for *I. ubique* compared to 1.2 for *I. dendrocinclae.* The sporocysts of *I. ubique* have no substieda bodies and contain a scattered residuum compared to the subglobular substieda bodies and condensed, subspherical residuum of *I. dendrocinclae. Isospora ocellati* and *I. striata* were described from the ocellated woodcreeper, *Xiphorhynchus ocellatus* (McQuistion *et al.* 1996). Although their oocysts are similar in size and shape compared to *I. dendrocinclae*, *I. striata* has striations around the anterior end of the sporozoites and rectangular shaped substeida bodies on the sporocysts while *I. dendrocinclae* has no striations on the sporozoites and subglobular shaped substieda bodies. Sporocysts of *Isospora ocellati* are shorter than *I. dendrocinclae* (11 x 8 µm vs. 12.9 × 8.3 µm) with a dome-like Stieda body, ellipsoidal substieda body, and scattered residuum compared to the knoblike Stieda bodies, subglobular substieda bodies and compact, subspherical sporocyst residuum of *I. dendrocinclae.*

Acknowledgements. The authors thank Ms Aidan Parrish for the line drawing and the Academy of Natural Sciences of Philadelphia for funding the bird collecting expedition.

REFERENCES

Barta J. R., Coles B. A., Schito M. L., Fernando M. A., Martin A., Danforth H. D. (1998) Analysis of infraspecific variation among five strains of *Eimeria maxima* from North America. *International J. Parasitol*. **28:** 485–492

Barta J. R. (2001) Molecular approaches to inferring evolutionary relationships among protistan parasites. *Vet. Parasitol*. **101:** 175–186

Brown L. M., Ramey II R. R., Tamburini B., Gavin T. A. (2004) Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. *Conserv. Genet.* **5:** 743–757

Capparella A. P. (1988) Genetic variation in Neotropical birds: Implications for the speciation process. *Acta Congressus Internationalis Ornithologici* **19:** 1658–1664

del Hoyo J., Elliott A., Christie D. A. eds. (2003) Handbook of the Birds of the World. Vol. 8: Broadbills to Tapaculos. Lynx Editions, Barcelona, Spain

McQuistion T. E., Wilson M. (1989) *Isospora geospizae*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the small ground finch (*Geospiza fuliginosa*) and the medium ground finch (*Geospiza fortis*) from the Galapagos Islands. *Syst. Parasitol.* **14:** 141–144

McQuistion T. E., Capparella A. P. (1995) Two new isosporan parasites (Apicomplexa: Eimeriidae) from the South American woocreeper *Dendrocolaptes certhia*. *Acta Protozool*. **34:** 299–302

McQuistion T. E., Walden K. K., Capparella A. P. (1996) Two new species of *Isospora* (Apicomplexa: Eimeriidae) from the ocellated woodcreeper, *Xiphorhynchus ocellatus* (Passeriformes: Dendrocolaptidae). *Arch. Protistenk*. **147:** 401–404

McQuistion T. E., Capparella A. P. (1997) *Isospora ubique*: A new coccidian parasite from the wedge-billed woodcreeper (*Glyphorynchus spirurus*) from South America. *Acta Protozool*. **36:** 75–78

Peters J. L. (1951) Check-list of Birds of the World. Vol. VII. Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

Ridgley R. S., Tudor G. (1994) The Birds of South America. University of Texas Press, Austin, Texas

Received on 25th September, 2009; revised on 8th March, 2010; accepted on 13th March, 2010