

## The complete mitochondrial genome sequences of two *Isospora* species (Eimeriidae, Eucoccidiorida, Coccidiasina, Apicomplexa) causing coccidiosis in superb glossy starlings, *Lamprotornis superbus* (Aves: Sturnidae)

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### ABSTRACT

Complete mitochondrial genomes are reported for two *Isospora* species causing systemic coccidiosis in Superb Glossy Starlings (Aves: Sturnidae). The A/T rich (34.7% G/C) genomes were 6223 bp in length for *Isospora greineri* and 6217 bp for *Isospora superbusi*. Each encoded 3 protein-coding genes, (COI, COIII and CytB) plus 18 LSU and 14 SSU rDNA fragments. Arrangement of protein- and rRNA-coding regions was identical to known *Eimeria* sp. mt genomes; start codon usage was conventional. The mitochondrial genome structures of *Isospora* and *Eimeria* species are conserved and reflect the close phylogenetic association between these eimeriid genera of apicomplexan parasites.

### ARTICLE HISTORY

Received 31 October 2017  
Accepted 17 November 2017



### KEYWORDS

Coccidia; fragmented rRNA;  
*Isospora*; parasitology;  
atoxoplasmosis

Passeriformes (e.g. canaries, finches, sparrows, grosbeaks and starlings) and other birds are commonly parasitized by coccidia transmitted via the fecal-oral route (Box 1981; Levine 1982; Schrenzel et al. 2005). More than 90% of all the described coccidia infecting wild birds belong to the genus *Isospora* (see Pellérdy 1974). In passeriform birds, enteritis caused by *Isospora* spp. resembles coccidiosis in poultry and subclinical infections are common (Page and Haddad 1995). Systemic (extraintestinal) coccidiosis is caused by some *Isospora* species that migrate during merogony (Quiroga et al. 2000; Upton et al. 2001; Cushing et al. 2011; Hafeez et al. 2014). Host specificity of *Isospora* species is believed to be relatively narrow, perhaps at the host genus level, like many other eimeriid coccidia (Long 1982). Levine (1982) generalized that a particular coccidium is likely to be transmissible between host species within the same host genus but not to a different host genus, even if in the same family.

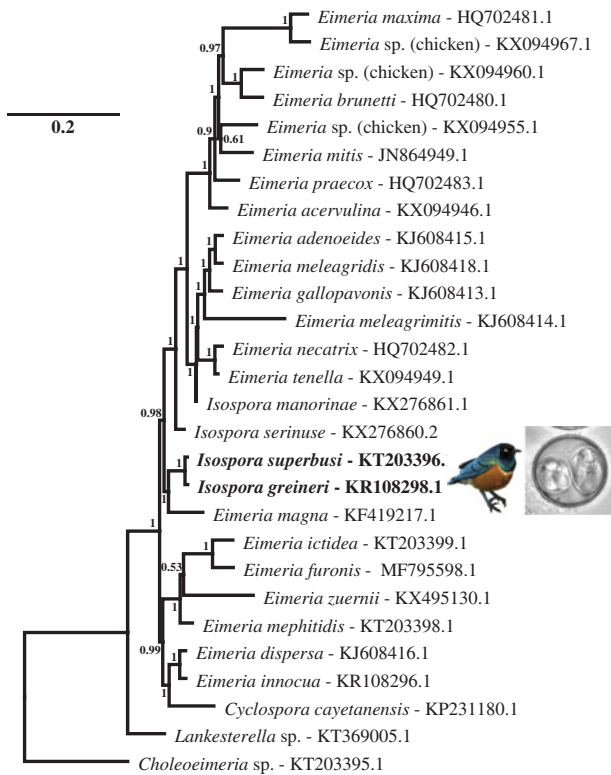
Specific identification based on microscopy is almost impossible because some *Isospora* species possess morphologically indistinguishable oocysts (Hafeez et al. 2014). Molecular data from avian *Isospora* species are scarce and, where available, are usually nuclear small subunit rDNA sequences (Carreno and Barta 1999). Schrenzel et al. (2005) also obtained short mt COI sequences in one of the first such uses of mt sequences in molecular studies of these coccidia. In the present study, the complete mitochondrial genome sequences are reported for two recently named *Isospora* spp. (see Hafeez et al. 2014) that caused systemic coccidiosis in captive Superb Glossy Starlings (*Lamprotornis superbusi*; Aves: Sturnidae) held at the Toronto Zoo (43.819583, -79.184722).

DNA was extracted from oocysts and infected tissues containing numerous merozoites obtained during diagnostic necropsy (liver, spleen, lungs and intestine) as described (Hafeez et al. 2014) and the mt genome amplified using long-range PCR with coccidia-specific primers to obtain complete mt genomes from *Isospora greineri* (6223bp) and *Isospora superbusi* (6217bp) assembled using de novo sequence assembly within Geneious software (Version 6.1 and later, from <http://www.geneious.com>). The mitochondrial genomes of *I. greineri* (GenBank: KP108298.1; Canadian Museum of Nature hepatotype series CMNPA 2014-0002) and *I. superbusi* (GenBank: KT203396.1; Canadian Museum of Nature hepatotype series CMNPA 2014-0003) had three protein-coding genes (COI, COIII and CytB) as well as 18 LSU and 14 SSU rDNA fragments but no regions encoding tRNAs. The two *Isospora* spp. had 99.2% pairwise sequence identity with the majority of differences within the protein-coding or intergenic regions (Figure 1). Genome organization was identical to that observed in a variety of eimeriid mt genomes (Ogedengbe et al. 2014); start codon usage, start positions and TAA termination codons for COI, COIII and CytB were consistent with those documented previously for other coccidian mt genomes (Ogedengbe et al. 2013). Our amplification of overlapping PCR products covering the complete mt genome suggests that the *Isospora* spp. mt genome is physically either circular (like *Plasmodium* spp.; Feagin et al. 2012) or a concatemer of multiple genome copies; the latter form is probable because concatenated mt genomes are reported for closely related *Eimeria* spp. (see Hikosaka et al. 2010).

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**Figure 1.** Phylogenetic tree based on the protein- and rRNA-coding regions of complete mitochondrial genome sequences from a variety of eimeriid coccidia demonstrates the close relationship between *Isospora superbusi* and *Isospora greineri*. The Bayesian analysis was performed on an alignment of 5394bp from each complete mitochondrial genome from the 3 CDS and 32 rDNA fragments; the dataset was partitioned so that the CDS were analyzed using a codon-based (mtmet translation) substitution model and the rDNA regions analyzed using a GTR + G + I substitution model. Scale bar indicates hypothesized evolutionary divergence and numbers at nodes indicate Bayesian posterior probabilities. Superb Glossy Starling image was modified from the original of Sumeet Moghe and used under license CC BY-SA 3.0.

## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

## Funding

This research was supported by a Discovery Grant (400566) to JRB from the Natural Sciences and Engineering Research Council of Canada (NSERC).

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