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A new species of *Eimeria* (Apicomplexa: Eimeriidae) from brown anole, *Anolis sagrei* (Sauria: Dactyloidae) from Florida, USA

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Abstract

During July 2014, 14 brown anoles, *Anolis sagrei* Duméril and Bibron were collected from Orange County, Florida, U.S.A., and their faeces examined for coccidian parasites. One (7%) harboured an eimerian that we describe here as new. Oöcysts of *Eimeria garmani* sp. n. were ellipsoidal with a uni-layered wall and measured (length $\times$ width, L $\times$ W) 19.3 $\times$ 12.5 μ m, with a length/width (L/W) ratio of 1.5. A micropyle, oöcyst residuum and polar granule were absent. Sporocysts were subspheroidal, 6.8 $\times$ 6.3 μ m, L/W 1.1. Stieda, substieda and parastieda bodies were absent. A sporocyst residuum was present as dispersed granules. Endogenous stages were observed within the small intestine. This is the first coccidian reported from the brown anole and the third eimerian reported from anoles in the United States.

Introduction

The brown anole, *Anolis sagrei* Duméril and Bibron, is a brown to gray lizard that is native to Cuba, the Isla de Juventud, the Bahamas, Swan Island, and the Islas de la Bahia, Honduras (Schwartz & Henderson, 1991); it has been introduced into 13 US states and is now established in Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, South Carolina and Texas (Conant & Collins, 1998; Parmley 2002; McAllister et al., 2003; Crother et al., 2012; Dixon 2013). Around 1887, brown anoles came to southern Florida (Garman, 1887) and, more recently, have colonized Hawaii (Goldberg et al., 2000), Jamaica (Bundy et al., 1987), and Taiwan (Norval et al., 2011). Brown anoles primarily feed on insects but they also consume lizards, including smaller conspecifics, native green anoles, *Anolis*

carolinensis Voigt and nonindigenous red-sided curly-tailed lizards, *Leiocephalus schreibersii* (Gravenhorst) in Florida (Krysko & Wasilewski, 2013).

Although a great deal has been published on their helminth parasites (Bundy et al., 1987; Goldberg et al., 1994, 2002; Goldberg & Bursey, 2000; Norval et al., 2011; Langford et al., 2013), nothing is known of coccidia in these lizards (McAllister et al., 2014). Herein, we provide a description of an eimerian from *A. sagrei* from Florida.

Materials and methods

During July 2014, 14 juvenile and adult *A. sagrei* were collected from Orange County, Florida, USA, and their faeces examined for coccidian parasites. Faecal samples from the rectum were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Once they had completely sporulated, measurements were taken on 24 oöcysts from a single anole using a calibrated ocular micrometer or Olympus® cellSens 1.7 digital software and reported in micrometres (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oöcysts were c. 110 days old when measured and photographed. Tissue samples from the small intestine and gall bladder of a single infected anole were fixed in 10% neutral-buffered formalin and processed as histological sections (at 6 μm) following standard methods of staining with hematoxylin and eosin.

In an attempt to rupture oöcyst walls and subsequently release sporocysts and their sporozoites from sporocyst valve sutures, 150 oöcysts in ATL lysis buffer were subjected to 600 mAU/ml proteinase K enzyme (DNA Easy Blood and Tissue Kit, Qiagen, Hilden, Germany) following the manufacturer's instructions. Oöcysts were incubated at 56 C for 2, 6, 12 and 24 hr and, immediately after each time period, samples were placed on a microscopic slide and inspected by light microscopy under oil immersion at 1,000 $\times$. We also did another technique by freezing the oöcysts for 6 hours at -80 C followed by boiling for 5 minutes. Again, samples were inspected at 1,000 $\times$. Because this resulted in about 80% of oöcysts being shattered, we repeated this experiment hoping that the remaining intact oöcysts would gradually open, releasing sporocysts.

A host voucher was accessioned into the Henderson State University Collection (HSU), Arkadelphia, Arkansas, U.S.A. Photosyntypes of sporulated oöcysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, U.S.A. Scientific names of reptiles follow the TIGR Reptile Database (Uetz & Hošek, 2014).

Results

One (7%) of the *A. sagrei* was found to be passing oöcysts of a new species of coccidian, which is described below. Unfortunately, attempts at releasing sporozoites from sporocyst sutures was not successful using our two experimental approaches.

***Eimeria garmani* n. sp**

Type-host: Brown anole, *Anolis sagrei* Duméril and Bibron, 1837 (Reptilia: Sauria: Dactyloidae) (adult male, symbiotype HSU 1801 collected 22.vii.2014).

Type-locality: 24 km SW Orlando, Orange County, Florida, USA (28.352785°N, 81.540483°W).

Type-material: Photosyntype deposited in the HWML 75132.

Prevalence: In 1 of 14 (7%) of the type host.

Sporulation time: Exogenous. All oöcysts were passed unsporulated or partially sporulated and fully sporulated within 5 days at c.23°C.

Site of infection: Small intestine (Figs. 5–6). Sections of gall bladder tissue (Figs. 7–8) did not reveal endogenous stages suggesting a *Choleoeimeria* sp.

Etymology: The specific epithet is given in honor of Samuel Walton Garman (1843–1927), American zoologist and naturalist, who first reported *A. sagrei* in the U.S.A. in 1887. He was also the first official curator of fishes, amphibians and reptiles at the Museum of Comparative Zoology, Harvard University, Boston, Massachusetts, U.S.A.

Description (Figs. 1–6)

Sporulated oöcyst: Oöcyst (n = 24) colourless, smooth, ellipsoidal; 19.3×12.5 (17–22 $\times$ 12–14), length/width (L/W) ratio 1.5 (1.3–1.7). Wall single-layered, c.0.6 thick. Micropyle absent, oöcyst residuum absent, 1 (bi-lobed), rarely 2 polar granule(s) present.

Sporocyst: Sporocysts (n = 24) four, colourless, smooth, subspheroidal, 6.8×6.3 (6–8 $\times$ 5–7); L/W ratio 1.1 (1.0–1.2); wall single-layered c.0.5 thick, with valves joined by longitudinal sutures. Stieda body, sub-Stieda, and para-Stieda bodies absent; sporocyst residuum composed of large-sized granules in compact mass between sporozoites.

Sporozoite: Sporozoites 2, sausage-shaped, not measured; single subspheroidal anterior refractile body and subspheroidal posterior refractile body, with nucleus slightly posterior to midpoint.

Remarks—Because we observed endogenous stages in the small intestine (Figs. 5–6) and did not observe endogenous stages developing in hypertrophied, displaced cells of the gall bladder or biliary epithelium (Figs. 6–7) (see Jirk et al., 2002), we placed the new species in the genus *Eimeria* rather than *Choleoeimeria* (*sensu* Paperna & Landsberg, 1989). Even in the absence of distinct DNA sequences that separate these two genera (Jirk et al., 2002), we feel comfortable with this placement.

When the new species is compared to other ellipsoidal eimerians described from other *Anolis* spp., it is most similar to *Eimeria intermedia* Ruiz, 1959 from the intermediate anole, *Anolis intermedius* Peters from Costa Rica (Ruiz, 1959; McAllister et al., 2014). However, oöcyst width of the new species is considerably smaller than *E. intermedia* (12.5 vs. 14.9

µm). It further differs by having subspheroidal to ovoidal sporocysts compared to the spheroidal sporocysts of *E. intermedia* as well as having polar granules that *E. intermedia* does not possess.

Discussion

McAllister et al. (2014) recently provided a summation of the coccidia of the lizard family Dactyloidae that included 17 species of coccidians from 10 *Anolis* spp. in the Western Hemisphere. The number of coccidians known to date is obviously a large underestimate of the total worldwide diversity as only 2.5% of the 395 species of *Anolis* (Uetz & Hošek 2014) have been reported to harbor coccidia and no one really knows how many have ever been examined in the past but not found to be passing oocysts. Within the United States alone, only native *A. carolinensis* has been reported to host eimerians, with *Eimeria anolidis* Daszak & Ball, 1991 from Florida (which actually may be a *Choleoeimeria*), and *Eimeria robisoni* McAllister, Seville, & Connior, 2014 from Arkansas. Prior to our study, none of the 10 exotic species of *Anolis* within the United States had ever been examined, to our knowledge, for coccidia, and here we describe the first coccidian from one of these introduced species. The establishment of *A. sagrei* within Florida is from multiple introductions, leading to a genetic structure of Florida populations that is unique from native populations within Cuba (Kolbe et al., 2004). It would be interesting to see if this new coccidian occurs within the native range of *A. sagrei* or of native *A. carolinensis* in Florida. We suggest additional surveys on other exotic *Anolis* in order to determine the geographic distribution, diversity, and abundance of coccidia within the continental United States.

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Figs. 1–3.

Nomarski interference-contrast photomicrographs of oocysts of *Eimeria garmani* n. sp. 1, Ellipsoidal oöcyst and sporocyst (SP) and sporocyst residuum (SR). 2, Oöcyst showing polar granule (PG). 3, Individual sporocyst showing suture (SU). *Scale bars:* Figs. 1–2, 10 μm ; Fig. 3, 2 μm .

Fig. 4.
Composite line drawing of oocyst of *Eimeria garmani* n. sp. Scale bar: 5 μ m.

Figs. 5–6.

Endogenous stages of *Eimeria garmani* n. sp. in columnar epithelium of small intestine. 5, Mature meront (ME) containing 6–7 merozoites. 6, Developing meront (ME) enclosed within parasitophorous vacuole. *Scale bars:* 10 μ m.

Figs. 7–8.

Gallbladder sections. 7, Whole gallbladder. *Scale bar:* 100 μm . 8, Higher magnification of gallbladder showing only sloughed epithelial cells. *Scale bar:* 10 μm .