

A new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from the brown anole *Anolis sagrei* Duméril & Bibron (Sauria: Dactyloidae) in Florida, USA

Chris T. McAllister · R. Scott Seville ·
Matthew B. Connior · Stanley E. Trauth

Received: 9 December 2014 / Accepted: 18 March 2015
© Springer Science+Business Media Dordrecht 2015

Abstract During July 2014, 14 brown anoles, *Anolis sagrei* Duméril & Bibron were collected from Orange County, Florida, USA, and their faeces examined for coccidian parasites. One (7%) lizard harboured an eimerian that we describe here as new. Oöcysts of *Eimeria garmani* n. sp. were ellipsoidal with a unilayered wall and measured $19.3 \times 12.5 \mu\text{m}$, with a length/width ratio of 1.5. A micropyle and oöcyst residuum were absent but 1–2 polar granule(s) were present. Sporocysts were subspheroidal, $6.8 \times 6.3 \mu\text{m}$, with a length/width ratio of 1.1. Stieda, sub-Stieda and para-Stieda 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 & 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 Bahía, 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 colonised Hawaii (Goldberg & Bursey, 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).

C. T. McAllister (✉)
Science and Mathematics Division, Eastern Oklahoma
State College, Idabel, OK 74745, USA
e-mail: cmcallister@se.edu

R. S. Seville
Department of Zoology and Physiology, University of
Wyoming, Casper, WY 82601, USA

M. B. Connior
Life Sciences, Northwest Arkansas Community College,
Bentonville, AR 72712, USA

S. E. Trauth
Department of Biological Sciences, Arkansas State
University, State University, AR 72467, USA

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 with ranges followed by the means 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×. We also tried 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×. 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,

USA. 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 & Bibron (Reptilia: Sauria: Dactyloidae) (adult male, symbiotype HSU 1801 collected 22.vii.2014).

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

Type-material: Photosyntype deposited in the HWML 75132.

Prevalence: 7% (in 1 of 14 specimens 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 USA in 1887. He was also the first official curator of fishes, amphibians and reptiles at the Museum of Comparative Zoology, Harvard University, Boston, Massachusetts, USA.

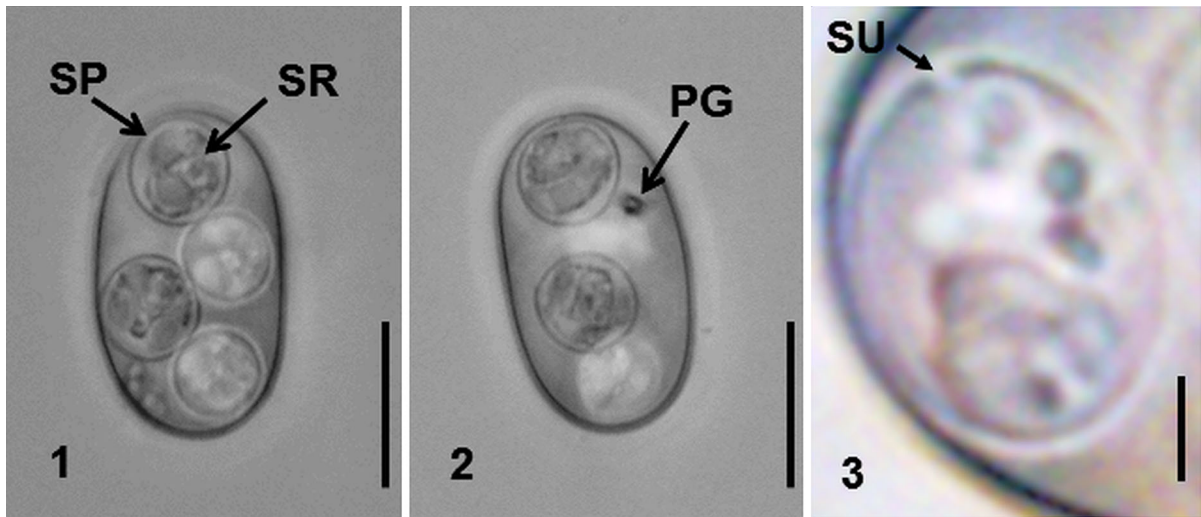
Description (Figs. 1–6)

Sporulated oöcyst

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

Sporocyst

Sporocysts (n = 24) 4, colourless, smooth, subspheroidal, 6–8 × 5–7 (6.8 × 6.3); L/W ratio 1.0–



Figs. 1–3 Nomarski interference-contrast photomicrographs of oöcysts 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: 1–2, 10 µm; 3, 2 µm

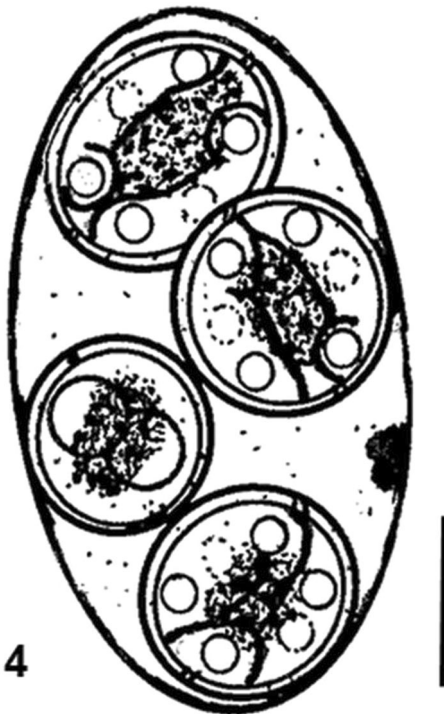


Fig. 4 Composite line drawing of oöcyst of *Eimeria garmani* n. sp. Scale-bar: 5 µm

1.2 (1.1); wall single-layered *c.*0.5 thick, with valves joined by longitudinal sutures. Stieda, sub-Stieda, and para-Stieda bodies absent; sporocyst residuum

composed of large-sized granules in compact mass between sporozoites.

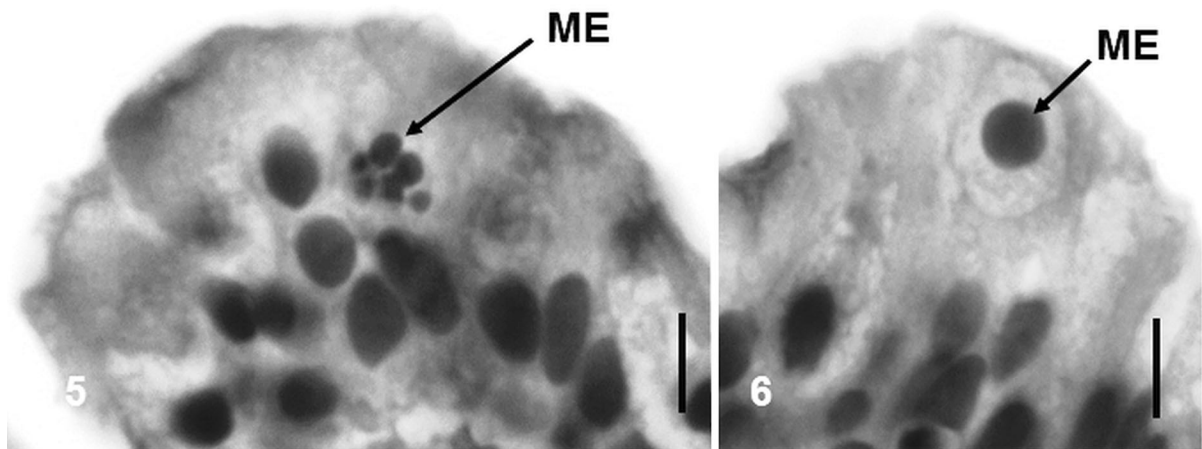
Sporozoite

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

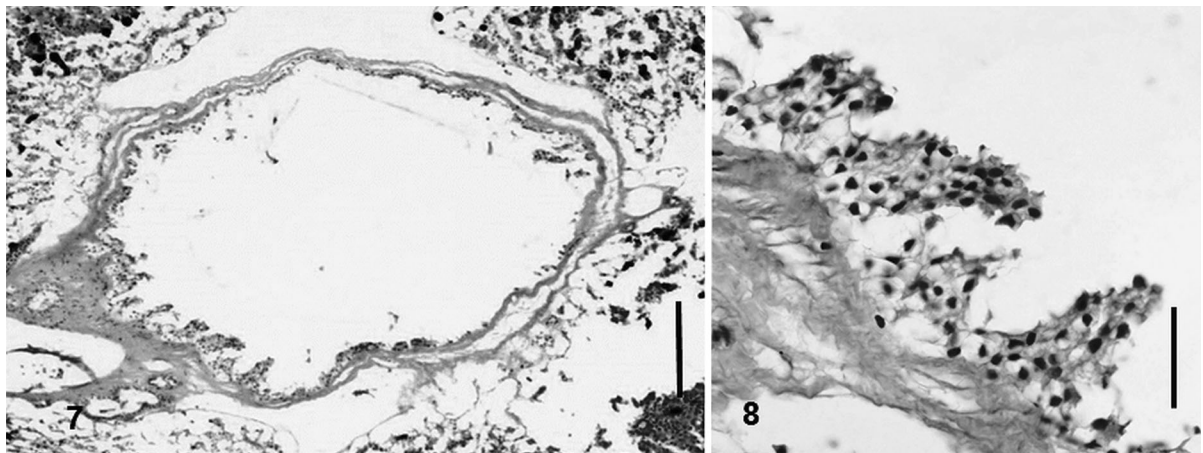
Remarks

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. 7–8) (see Jirků et al., 2002), we placed the new species in the genus *Eimeria* Schneider, 1875 rather than *Choleoimeria* Paperna & Landsberg, 1989 (see Paperna & Landsberg, 1989). Even in the absence of distinct DNA sequences that separate these two genera (Jirků 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



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 Gall bladder sections. 7, Whole gall bladder. 8, Higher magnification of gall bladder showing only sloughed epithelial cells. Scale bars: 7, 100 μ m; 8, 10 μ m

14.9 μ m). 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 ten *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* (see Uetz & Hošek, 2014) have been reported to harbour coccidia and no one really knows how many have ever been examined in the past but not found to be passing oöcysts. 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* spp. in order to determine the geographic distribution, diversity, and abundance of coccidia within the continental United States.

Acknowledgements We thank Drs. S. L. Gardner (HWML) and R. Tumlison (HSU) for expert curatorial assistance and Dagmara Motriuk-Smith (Univ. Wyoming) for technical assistance.

Funding This study was supported, in part, by a grant from the National Institute of General Medical Sciences (8P20GM103432-12), National Institutes of Health to R.S. Seville. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethical standards All applicable institutional, national and international guidelines for the care and use of animals were followed. A scientific collecting permit was provided to MBC by the Florida Fish and Wildlife Conservation Commission (permit number LSSC 13-00033).

References

- Bundy, D. A. P., Vogel, P., & Harris, E. A. (1987). Helminth parasites of Jamaican anoles (Reptilia: Iguanidae): A comparison of the helminth fauna of 6 *Anolis* species. *Journal of Helminthology*, *61*, 77–83.
- Conant R., & Collins J. T. (1998). *A field guide to reptiles and amphibians of eastern and central North America*, 3rd ed. (expanded). Boston: Houghton Mifflin, 616 pp.
- Crother, B. I. [Committee Chair]. (2012). Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. *Society for the Study of Amphibians and Reptiles Herpetological Circular*, *39*, 1–101.
- Dixon, J. R. (2013). *Amphibians and reptiles of Texas: With keys, taxonomic synopses, bibliography, and distribution maps*. College Station: Texas A&M University Press, 447 pp.
- Garman, S. (1887). On West Indian Iguanidae and on West Indian Scincidae. *Bulletin of the Essex Institute*, *19*, 1–29.
- Goldberg, S. R., & Bursey, C. R. (2000). Transport of helminths to Hawaii via the brown anole, *Anolis sagrei* (Polychrotidae). *Journal of Parasitology*, *86*, 750–755.
- Goldberg, S. R., Bursey, C. R., & Tawil, R. (1994). Helminth parasites of the bark anole, *Anolis distichus* and the brown anole, *Anolis sagrei* (Polychridae) from Florida and the Bahamas. *Caribbean Journal of Science*, *30*, 275–277.
- Goldberg, S. R., Bursey, C. R., & Kraus, F. (2002). Seasonal variation in the helminth community of the brown anole, *Anolis sagrei* (Sauria: Polychrotidae), from Oahu, Hawaii. *American Midland Naturalist*, *148*, 409–415.
- Jirků, M., Modrý, D., Šlapeta, J. R., Koudela, B., & Lukes, J. (2002). The phylogeny of *Goussia* and *Choleoemimeria* (Apicomplexa: Eimeriorina) and the evolution of excystation structures in coccidia. *Protist*, *153*, 379–390.
- Kolbe, J. J., Glor, R. E., Schettino, L. R., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, *431*, 177–181.
- Krisko, K. L., & Wasilewski, J. A. (2013). Natural history notes: *Anolis sagrei*. *Herpetological Review*, *43*, 477–478.
- Paperna, I., & Landsberg, J. H. (1989). Description and taxonomic discussion of eimerian coccidia from African and Levantine geckoes. *South African Journal of Zoology*, *24*, 245–355.
- Langford, G. J., Willobee, B. A., & Isidoro, L. F. (2013). Transmission, host specificity, and seasonal occurrence of *Cyrtosomum penneri* (Nematoda: Atractidae) in lizards from Florida. *Journal of Parasitology*, *99*, 241–246.
- McAllister, C. T., Trauth, S. E., & Harris, C. S. (2003). Geographic distribution: *Anolis sagrei*. *Herpetological Review*, *34*, 261–262.
- McAllister, C. T., Seville, R. S., & Connior, M. B. (2014). A new caryosporan and eimerian (Apicomplexa: Eimeriidae) from green anoles, *Anolis carolinensis* (Sauria: Dactyloidae), from Arkansas and Louisiana, with a summary of the coccidia of the Dactyloidae. *Journal of Parasitology*, *100*, 480–484.
- Norval, G., Bursey, C. R., Goldberg, S. R., Mao, J. J., & Slater, K. (2011). Origin of the helminth community of an exotic invasive lizard, the brown anole, *Anolis sagrei* (Squamata: Polychrotidae), in southwestern Taiwan. *Pacific Science*, *65*, 383–390.
- Parmley, D. (2002). Northernmost record of the brown anole (*Anolis sagrei*) in Georgia. *Georgia Journal of Science*, *60*, 191–193.
- Ruiz, A. (1959). *Eimeria intermedia* n. sp., parasita de la lagaraja *Anolis intermedius* Peters. *Revista de Biología Tropical*, *7*, 109–112.
- Schwartz, A., & Henderson, R. W. (1991). *Amphibians and reptiles of the West Indies: Descriptions, distributions, and natural history*. Gainesville: University of Florida Press, 720 pp.
- Uetz, P., & Hošek, J. (2014). The TIGR Reptile Database. World Wide Web electronic publication. <http://www.reptile-database.org/>. Accessed 6 December 2014.