Published in final edited form as:

. 2014 July 1; 81(2): 175–178. doi:10.1654/4665.1.

A New Species of *Eimeria* (Apicomplexa: Eimeriidae) from Green Frog, *Lithobates clamitans* (Anura: Ranidae) from Arkansas, U.S.A

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Abstract

Between April and October 2012, 20 juvenile and adult green frogs (*Lithobates clamitans*) were collected by hand or dipnet from 3 counties of Arkansas and examined for coccidial parasites. A single frog (5%) was found to be passing oocysts of a new eimerian species. Oocysts of *Eimeria menaensis* n. sp. were ellipsoidal to subspheroidal with a bilayered wall and measured (L × W) 25.4 × 15.6 (23–27 × 13–17) μm, with a L/W ratio of 1.6. A micropyle was absent but an oocyst residuum and polar granule were present. Sporocysts were spheroidal to subspheroidal and measured 5.0 × 5.0 (4–6) μm with L/W of 1.1. An indistinct Stieda body was present, but sub- and para-Stieda bodies were absent. The sporocyst residuum consisted of condensed granules dispersed between sporozoites. Sporozoites were elongate and attenuated at both ends with spheroidal anterior and posterior refractile bodies. This represents the second report of coccidia from *L. clamitans* and the first time a coccidian has been reported from a green frog from Arkansas.

Keywords

Arkansas; Apicomplexa; bronze frog; coccidia; green frog; *Eimeria menaensis*; *Lithobates clamitans*

The green frog, *Lithobates clamitans* (Latreille, 1801) has an extensive range from the Canadian border south and west from Minnesota to eastern Texas and further east through the Gulf of Mexico to northern Florida and up the coast to Maine (Conant and Collins, 1998). In Arkansas, *L. clamitans* is found statewide with the nominate subspecies, *L. c. clamitans* (bronze frog) found in the Gulf Coastal Plain ecoregion and *L. c. melanotus* occupying the Interior Highland of the Ozark and Ouachita plateaus (Trauth et al. 2004). Much is known about the natural history of this frog including several studies on its parasites (see Pauley and Lannoo, 2005). Here we present a description of a new species of *Eimeria* from *L. clamitans* from Arkansas.

MATERIALS AND METHODS

Between April and October 2012, 20 juvenile and adult green frogs (mean $\pm$ 1SD snout–vent length [SVL] = 46.5 ± 12.9 , range 25–80 mm) were collected by hand or dipnet from 3 counties of Arkansas as follows: Benton ($n = 1$, *L. c. melanotus*), Polk ($n = 6$, *L. c. melanotus*) (Fig. 1) and Union ($n = 13$, *L. c. clamitans*). Specimens were placed in individual plastic bags on ice and transported to the laboratory within 48 hr for necropsy. Frogs were anesthetized with a concentrated chloretone solution and a mid-ventral incision was made to expose the visceral contents. Individual fecal samples from the rectum were placed in vials containing tap water supplemented with 100 IU penicillin–G/ml and 100 μ g streptomycin/ml (pen–strep). Samples were examined for coccidia using Brightfield microscopy following flotation in Sheather's sugar solution (sp. gr. = 1.30). A single positive sample containing unsporulated oocysts was placed in a Petri dish containing a thin layer of tap water supplemented with pen–strep solution. Following a sporulation period of 5 days at room temperature ($\sim 23^{\circ}\text{C}$), partially sporulated and fully sporulated oocysts were forwarded to RSS for further examination. Samples were again examined after flotation and measurements were taken on 10 oocysts using a calibrated ocular micrometer or Olympus® cellSens 1.7 digital imaging software and reported in micrometers (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were measured and photographed 11 days after being recovered from a single frog. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granules (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocysts (SP), Stieda body (SB), sub–Stieda body (SSB), para–Stieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N). Tissue samples from the intestine of the single infected frog was fixed in 10% neutral–buffered formalin and processed as histological sections following standard methods of staining with hematoxylin and eosin. Host vouchers were accessioned into the Arkansas State University Museum of Zoology, Herpetological Collection (ASUMZ), State University, Arkansas, U.S.A., as ASUMZ 32251–32252, 32264,

32271, 32387, 32392–39396). Photosyntypes of sporulated oocysts as well as other parasites were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. Amphibian taxonomy follows the amphibian species of the world database (Frost, 2011).

RESULTS

One of 20 (5%) *L. clamitans* was found to actively pass oocysts of a new eimerian. The description follows.

Apicomplexa: Eimeriidae

***Eimeria menaensis* n. sp.**

Figures 1–4

Description

Description of sporulated oocyst: Oocyst shape ellipsoidal; bilayered wall, ~0.7 thick, smooth outer layer ~0.4, inner layer ~0.3; L × W 25.4 × 15.6 (23–27 × 13–17), L/W 1.6 (1.5–1.8). M absent but prominent spheroidal OR (10.0) and single PG present.

Description of sporocyst and sporozoites: SP spheroidal to subspheroidal, with a smooth single-layered wall; L × W 5.0 × 5.0 (4–6); L/W 1.0 (0.9–1.1); indistinct SB present, SSB, PSB absent; SR consists of condensed granules dispersed between SZ. SZ elongate (attenuated at both ends) with spheroidal ARB and PRB; single N slightly posterior to midpoint of body.

Taxonomic summary

Type host: *Lithobates clamitans melanotus* (Rafinesque, 1820), green frog, symbiotype ASUMZ 32387, male, 80 mm SVL, collected 20 July 2012.

Other hosts: None.

Type specimens: Photosyntype deposited as USNPC 106351.

Type locality: USA: Arkansas, Polk County, 5.0 km N of Mena, off St. Hwy. 88 at Blue Haze Vista Pond, Polk County, Arkansas (34°37'33.79"N, 94°14'40.00"W).

Prevalence: 1 of 20 (5%) *L. clamitans* overall; 1 of 7 (14%) *L. c. melanotus*; 0 of 13 (0%) *L. c. clamitans*.

Sporulation: Exogenous. All oocysts were passed in feces unsporulated or partially sporulated and some fully sporulated within 5 days at ~23°C.

Prepatent and patent periods. Unknown.

Site of infection: Intestinal tract.

Endogenous stages: Development is extranuclear; we were only able to clearly observe the macrogamont stage (see Fig. 4).

Etymology: The specific epithet is given for the city of Mena (just south of the type locality), county seat of Polk County, Arkansas, which was incorporated in 1896. Mena is considered the gateway to some of the most visited tourist attractions in the state, including Rich Mountain, where the type host was collected.

Remarks

Eimeria menaensis should be placed within the tetrasporocystic coccidia “morphotype 1” group as defined by Jirk et al. (2009) which includes 7 other eimerians characterized with an OR, a small SB, and extranuclear endogenous development. Within this group only 2 species, *Eimeria streckeri* Upton and McAllister, 1988 and *Eimeria kermi* Chen and Desser, 1988 are known from North American anurans (*Pseudacris* spp., and *Lithobates* spp., respectively). The former, originally described from hylid frogs from Texas (Upton and McAllister, 1988) and later reported from Nebraska by Bolek et al. (2003), possesses spheroidal oocysts, and the latter, from Canadian ranid frogs, including *L. clamitans* (Chen and Desser, 1988), although similar in oocyst length, possesses wider oocysts (19.5 vs. 15.6 μ m) and a significantly smaller L/W index (1.3 vs. 1.6). One other species, *Eimeria algonquini* Chen and Desser, 1988, was previously reported from *L. clamitans* from Canada (Chen and Desser, 1998) but belongs to the “morphotype 3” group of Jirk et al. (2009). In addition, the only eimerian described previously from Arkansas anurans is *Eimeria fitchi* McAllister, Upton, Trauth, and Bursey, 1995, from wood frogs, *Lithobates sylvaticus* (McAllister et al., 1995); however, oocysts of *E. fitchi* belongs to the “morphotype 2” group of Jirk et al. (2009) and does not possess an OR, which is prominent in *E. menaensis* (see Fig. 1). The new species can easily be differentiated from all other eimerians reported from anurans (see Duszynski et al., 2007; Jirk et al., 2009).

In addition to 5 other *L. c. melanotus* and although sample sizes are not large, 8 additional frogs (all adults) collected on the same date at the type locality of *E. menaensis* were negative for coccidia, including 5 Cope’s gray treefrogs (*Hyla chrysoscelis*), 1 green treefrog (*Hyla cinerea*), and 2 southern leopard frogs (*Lithobates sphenoccephalus utricularius*).

DISCUSSION

Upton and McAllister (1988) were the first to provide a summary of the coccidia of the Anura of the world. Almost 2 decades later, Duszynski et al. (2007) provided a summation of the coccidia from all amphibians, and, more recently, Jirk et al. (2009) further extended our knowledge of eimerians from anurans. Including the new species described herein, only 19 species of *Eimeria* have been reported from anurans (Jirk et al., 2009, their Table 3). Therefore, together with isosporans from anurans and those species reported from salamanders (order Caudata) (Upton et al., 1993; Duszynski et al., 2007; McAllister and Upton, 2008), the coccidia of the class Amphibia are the most understudied vertebrate–host coccidian group. It is also obvious that some of these anuran coccidians (like *E. menaensis*) degrade quickly over a short period of time and should be studied as soon as sporulated

oocysts are obtained. This could be one reason that this group of vertebrates and their coccidia has been neglected from research. Additional study is suggested and more coccidians will likely be discovered in frogs and toads and even salamanders.

ACKNOWLEDGMENTS

We thank the Arkansas Game and Fish Commission and USDA Forest Service (Ouachita National Forest) for Scientific Collecting Permits issued to CTM, MBC and HWR. Further appreciation is extended to Patricia A. Pilitt (USNPC) for expert curatorial assistance, and Nikolas H. McAllister for assistance in collecting. This project was, in part, supported by grants from the National Center for Research Resources (5P20RR016474-12) and the National Institute of General Medical Sciences (8 P20 GM103432-12) from the National Institutes of Health to RSS.

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Figure 1.

Partially sporulated and completely sporulated oocysts of *Eimeria menaensis*. **1.** Partially sporulated oocyst showing distinct oocyst residuum (OR), polar granule (PG), indistinct Stieda body (SB) and sporocyst (SP). **2.** Completely sporulated oocyst showing anterior refractile body (ARB), posterior refractile body (PRB) and elongate sporozoite with attenuated ends (SZ). Scale bars = 10 µm.

Figure 2.
Composite line drawing of *Eimeria menaensis*. Scale bar = 10 μm.

Figure 3.
Photomicrograph of endogenous development of *Eimeria menaensis* in the intestinal tract of *Lithobates clamitans* showing various macrogamonts (arrows). Scale bar = 10 μ m.