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# Two new species of *Eimeria* (Apicomplexa, Eimeriidae) from tree skinks, *Prasinohaema* spp. (Sauria: Scincidae), from Papua New Guinea

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## Abstract

Between September 1991 and June 1992, feces from 4 species of tree skinks, *Prasinohaema* spp. from Papua New Guinea, were collected and examined for coccidia. Two species, *P. flavipes* and *P. prehensicauda* were found to harbor eimerians which are described as new. Oocysts of *Eimeria krausi* sp. nov. from *P. flavipes* were ellipsoidal to subspheroidal with a smooth bilayered wall and measured (L × W) 19.2 × 16.9 μm, with a length/width (L/W) ratio of 1.1. Micropyle and oocyst residuum were absent but a fragmented polar granule was present. Sporocysts were ellipsoidal, 9.7 × 6.7 μm, L/W of 1.5. Stieda, subStieda and paraStieda bodies were absent. The sporocyst residuum was composed of many small granules in a compact mass between sporozoites. The sporozoites were sausage-shaped, 11.7 × 2.7 μm, in situ, with an ellipsoidal posterior refractile body and a spheroidal anterior refractile body. Oocysts of *Eimeria greeri* sp. nov. from *P. prehensicauda* were ellipsoidal with a smooth bilayered wall, (L × W) 23.0 × 18.3 μm, with a L/W of 1.3. Micropyle and oocyst residuum were absent but a fragmented polar granule was present. Sporocysts were ellipsoidal, 9.7 × 8.4 μm, with a L/W of 1.2. Stieda, subStieda and paraStieda bodies were absent. The sporocyst residuum was composed of many large granules in a compact mass between sporozoites. The sporozoites were sausage-shaped, with an ellipsoidal posterior refractile body and a spheroidal anterior refractile body. We document here the first report of coccidia from skinks of the genus *Prasinohaema*.

## Keywords

Coccidia, *Eimeria greeri* sp. nov., *Eimeria krausi* sp. nov., *Prasinohaema flavipes*, *Prasinohaema prehensicauda*, Papua New Guinea, Sauria, Scincidae, tree skinks

## Introduction

Skinks of the genus *Prasinohaema* are an interesting group of New Guinean lizards that possess green blood plasma, the result of the accumulation of the bile pigment biliverdin (Austin and Jessing 1994). There are 5 named species which are restricted to New Guinea, adjacent islands along its southeastern peninsula, and the Solomon Islands (Greer 1974; Mys 1988). One species, *Prasinohaema flavipes* (Parker) inhabits high elevations in the central ranges of New Guinea, and the Huon Peninsula, having been recorded at 1,400 m on the latter and at 2,000 m in the Finisterre Range (Mys 1988). Another species, *Prasinohaema prehensicauda* (Loveridge) is known to inhabit the montane regions of the central highlands of Papua New Guinea (PNG), but is presumably absent from Indonesian New Guinea (Loveridge 1945).

There is little known about the parasites from this group of lizards. Austin and Perkins (2006) and Perkins and Austin (2009) looked at their haemoparasites and hypothesized that the unusual physiology of these lizards might reduce or preclude their infection by blood parasites. Goldberg *et al.* (2010) provided the only study, to date, of their metazoan endoparasites. Here, we document 2 new species of *Eimeria* from the intestinal contents of *Prasinohaema* spp. from PNG.

## Materials and Methods

Between September 1991 and June 1992, 4 species of *Prasinohaema* skinks ( $n = 14$ ), including 4 yellow-footed green-blooded skink, *P. flavipes*, 6 prehensile green tree skink, *P. prehensicauda*, 2 Semon's green tree skink, *P. semoni*

(Oudemans) and 2 green tree skink, *P. virens* (Peters) were collected by hand and fresh faeces from each was collected and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ( $K_2Cr_2O_7$ ). Upon return to the laboratory, each sample was examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 20 sporulated oocysts using a calibrated ocular micrometer and reported in micrometers ( $\mu\text{m}$ ) with means followed by the ranges in parentheses; photomicrographs were taken using Nomarski interference-contrast (NIC) optics. Oocysts were ~60 days old when measured and photographed. 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 (SP) 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). Voucher specimens of hosts were deposited in the Texas Natural History Collection (TNHC), Austin, Texas, USA. Photosyntypes of sporulated oocysts were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland. Lizard taxonomy follows the TIGR Reptile Database (Uetz and Hošek, 2013).

## Results

One of 6 (17%) *P. flavipes* and 1 of 4 (25%) *P. prehensicauda* were each passing oocysts of undescribed species of *Eimeria*, which are described below as new; 2 *P. virens* and 2 *P. semoni* were negative.

### *Eimeria krausi* sp. nov. (Figs 1–2, 4)

Description of sporulated oocyst: Oocyst shape: ellipsoidal to subspheroidal; colourless, smooth, bi-layered wall, ~1.4–1.6 thick, inner 0.4–0.6, outer 0.8–1.0; L  $\times$  W: 19.2  $\times$  16.9 (17–24

$\times$  15–20), L/W: 1.1 (1.0–1.2); M, OR absent, PG: present, but fragmented. Distinctive features of sporulated oocyst: Lack of M and OR, and sporocysts fill about  $\frac{2}{3}$  to  $\frac{3}{4}$  of space within the oocyst.

Description of sporocyst and sporozoites: SP shape: ellipsoidal, ~0.5 thick, with a smooth single-layered wall, L  $\times$  W: 9.7  $\times$  6.7 (9–11  $\times$  6–7), L/W 1.5 (1.3–1.8); SB, SSB, PSB, all absent: SR: present as a compact spheroidal mass, 3.5 (3–5) composed of many small granules between SZ; SZ: sausage-shaped, 11.7  $\times$  2.7 (10–13  $\times$  2–3) in situ, with ellipsoidal PRB, 3.0  $\times$  2.4 (2–4  $\times$  2–3) and spheroidal ARB, 1.6 (1–2); single N slightly posterior to midpoint of body. Distinctive features of sporocyst: lack of SB, SSB, PSB and the possible presence of a longitudinal suture that divides the SP into 2 walls.

Type host: Yellow-footed green-blooded skink, *Prasinohaema flavipes* (Parker, 1936) (Sauria: Scincidae); symbio-type host lost (Field Number CCA 0595) but others from same locality and date deposited in the TNHC 51882–4, 51958–65.

Type specimens: Photosyntype deposited as USNPC 107212.

Type locality: Kaironk Village, Madang Province, PNG (8°14'15"S, 144°28'50"E), elevation 2,000 m.

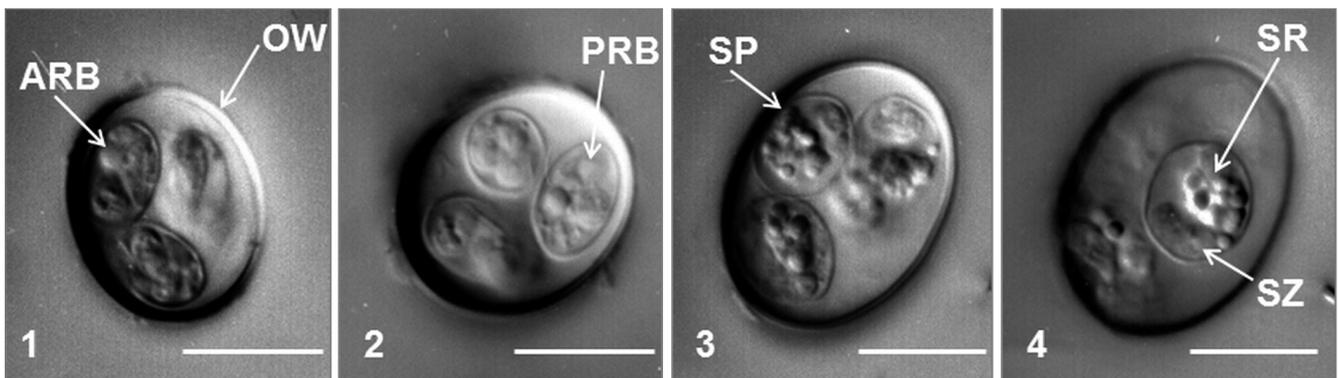
Prevalence: 1/6 (17%).

Sporulation: Unknown. Specimens were collected in the field, stored in  $K_2Cr_2O_7$  solution, and not examined until 60 days later.

Site of infection: Unknown. Oocysts were passed in faeces and host tissues were not collected or preserved for histological sectioning.

Etymology: The specific epithet is a patronym in honor of Dr. Fred Kraus, University of Michigan, Ann Arbor, Michigan, in recognition of his many contributions to the Papuan herpetofaunal literature.

Remarks: Sporulated oocysts of eimerians previously reported from skinks (see Modrý and Jirků 2006; McAllister *et al.* 2013b, c, d, *in press*) that are most similar in size to *E. krausi* include: *Eimeria leiolopismatis* Cannon, 1967 from the orange-tailed shadeskink, *Saproscincus challengerii* from Australia (Cannon, 1967) and *Eimeria lipinia* McAllister,



**Figs 1–4.** DIC photomicrographs of sporulated oocysts. 1–2. *Eimeria krausi* sp. nov. 3–4. *Eimeria greeri* sp. nov. Scale bars = 10  $\mu\text{m}$ . Abbreviations: ARB (anterior refractile body); OW (oocyst wall); PRB (posterior refractile body); SP (sporocyst); SR (sporocyst residuum); SZ (sporozoite)

Duszynski, Austin et Fisher, 2013 from the moth skink, *Lipinia noctua* from the Cook Islands, Fiji and PNG (McAllister *et al.* 2013c). However, sporulated oocysts of *E. krausi* can be differentiated from their oocysts as follows: oocysts of *E. krausi* are wider than *E. leiopismatis* (16.9 [15–20] vs. 15.7 [13–18]) and, although oocysts of *E. krausi* are similar in size to *E. lipinia* (19.2 × 16.9 [17–24 × 15–20], L/W = 1.1 vs. 18.6 × 16.9 [15–22 × 15–20], L/W = 1.1, see McAllister *et al.* 2013c) as are their SP (9.7 × 6.7 [9–11 × 6–7], L/W = 1.5 vs. 9.2 × 6.8 [8–11 × 6–8], L/W = 1.4, respectively, the SZ of *E. lipinia* contain only a PRB, whereas *E. krausi* SZs contains both PRB and ARBs. In addition to the host genus difference, behavioral, physiological and habitat differences between the hosts of these 3 forms suggest to us that they should be relegated to different species at this time. It appeared to us that there may be a suture in the SP wall, which could place *E. krausi* in another genus (*Acroeimeria*) except that species now placed into that genus are uniformly subspheroidal and undergo endogenous development epicytoplasmatically in the small intestine of infected hosts. Unfortunately, host tissues were not available for us to study; thus, until such material is available and/or until molecular evidence can suggest otherwise, we tentatively relegate this species to the genus *Eimeria*.

#### *Eimeria greeri* sp. nov. (Figs 3–4, 6)

Description of sporulated oocyst: Oocyst shape: ellipsoidal; colourless, smooth, bi-layered wall, ~1.2–1.6 thick, inner 0.4–0.6, outer 0.8–1.0; L × W: 23.0 × 18.3 (22–25 × 17–20), L/W: 1.3 (1.1–1.4); M, OR absent, PG: present, but fragmented. Distinctive feature of oocyst: lack of M and OR, and sporocysts fill only about 50% of the space within the oocyst.

Description of sporocyst and sporozoites: SP shape: ellipsoidal to subspheroidal, ~0.5 thick, with a single-layered wall; L × W: 9.7 × 8.4 (9–11 × 8–9), L/W 1.2 (1.1–1.2); SB, SSB, PSB, all absent: SR: present as a compact spheroidal mass

composed of many small granules between SZ; SZ: (not measured) elongate with ellipsoidal PRB and spheroidal ARB; single N slightly posterior to midpoint of body. Distinctive features of sporocyst: lack of SB, SSB, PSB and the possible presence of a suture that divides the SP into 2 walls.

Type host: Prehensile green tree skink, *Prasinochaema prehensicauda* (Loveridge, 1945) (Sauria: Scincidae). Collected 2 October 1991. Symbiotype TNHC 51870 (field number CCA 0596).

Type specimens: Photosyntype deposited as USNPC 107213.

Type locality: Kaironk Village, Madang Province, PNG (8°14'15"S, 144°28'50"E), elevation 2,000 m.

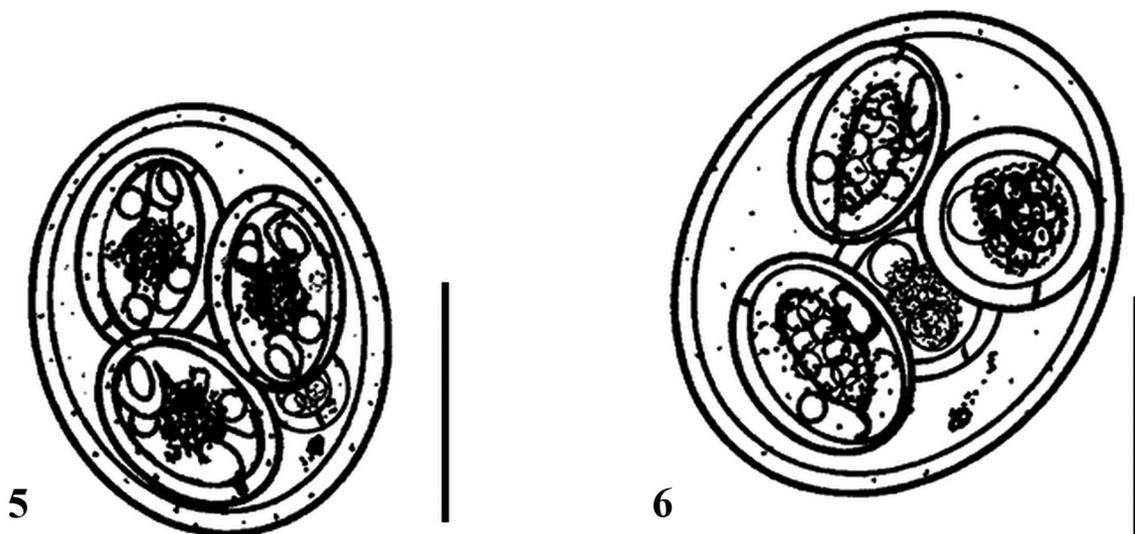
Prevalence: 1/4 (25%).

Sporulation: Unknown. Specimens were collected in the field, stored in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution, and not examined until 60 days later.

Site of infection: Unknown. Oocysts were passed in faeces and host tissues were not collected or preserved for histological sectioning.

Etymology: The specific epithet is a patronym in honor of Dr. Allen E. Greer, Australian Museum, in recognition of his many contributions to New Guinea skink taxonomy.

Remarks: Oocysts of *E. greeri* are most similar to those of *Eimeria nuailan* McAllister, Seville, Duszynski, Bush, Fisher et Austin, 2013 from emerald tree skinks, *Lamprolepis smaragdina* from PNG (20.3 × 18.3 [22–25 × 17–20], L/W = 1.1 vs. 23.7 × 19.1 [21–26 × 17–22], L/W = 1.3, McAllister *et al.* 2013d); however, SP of *E. greeri* are slightly smaller in length but larger in width with a significantly smaller L/W ratio (9.7 × 8.4 [9–11 × 8–9], L/W = 1.2 vs. 11.9 × 7.0 [10–14 × 6–8], L/W = 1.7). Just as in the previous description of *E. krausi*, there may be a suture in the SP walls of *E. greeri*, which also could place it in *Acroeimeria*; unfortunately, host tissues were not available for us to study; thus, until such material is available and/or until molecular evidence can suggest



Figs 5–6. Composite line drawings of oocysts. 5. *Eimeria krausi* sp. nov. 6. *Eimeria greeri* sp. nov. Scale bars = 10 µm

otherwise, we tentatively also relegate this species to the genus *Eimeria*.

## Discussion

The genus *Prasinohaema* is poorly known but is widespread in PNG, both in the lowlands and montane regions up to over 2,500 m; one lowland New Guinea species (*P. virens*) also occurs in the Solomon Islands. The species of this genus were described initially as a single entity, in part, due to the shared character of green blood (Greer 1974), but some of the species contain several other surprising characters for skinks such as adhesive “toe pads” (Irschick *et al.* 1996). All species in the genus are arboreal and, thus, are difficult to observe which may be why so little is known about their ecology and behavior. Two species, however, have color pattern sexual dimorphism (*P. prehensicauda* and *P. virens*) suggesting sexual selection is a force acting on the behavior in these 2 species (Kraus 2010). Very little is known about the parasite fauna of these green blooded lizards, and here we describe 2 new species of coccidia from this genus. Similar to other genera of southwest Pacific skinks recently surveyed (McAllister *et al.* 2013a, b, c, d, *in press*), these coccidia appear to be endemic to this genus. Both of these new *Eimeria* are most morphologically similar to species recently described from other arboreal genera of skinks, *Lipinia* and *Lamprolepis*, but until molecular studies can be done, we cannot make statements about their relationships. Further studies on coccidia within *Prasinohaema* and other genera in the southwest Pacific should continue to provide new species, and once more extensive surveys are completed, our understanding of the biogeography of these parasites will become clearer.

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