Two new species of Eimeria *Schneider, 1875 (Apicomplexa: Eimeriidae) from emerald tree skinks,* Lamprolepis smaragdina *(Lesson) (Sauria: Scincidae) from Papua New Guinea and the Philippines*

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Two new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from emerald tree skinks, *Lamprolepis smaragdina* (Lesson) (Sauria: Scincidae) from Papua New Guinea and the Philippines

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Abstract Two new species of *Eimeria* Schneider, 1875, from emerald tree skinks, *Lamprolepis smaragdina* (Lesson) are described from specimens collected in Papua New Guinea (PNG) and the Philippines. Oöcysts of *Eimeria nuiailan* n. sp. from the only *L. smaragdina* from PNG are ovoidal, with a smooth, colourless, bi-layered wall, measure 23.7 × 19.1 μm, and have a length/width (L/W) ratio of 1.3; both micropyle and oöcyst residuum are absent, but a fragmented polar granule is present. Sporocysts are ovoidal to ellipsoidal, 11.9 × 7.0 μm, L/W 1.7, and the wall is composed of two valves joined by a longitudinal suture; neither Stieda nor sub-Stieda bodies are present; a sporocyst residuum is present as a compact mass of granules. Sporozoites are elongate, 14.6 × 2.6 μm, and contain anterior and posterior refractile bodies with a nucleus between them. Oöcysts of *Eimeria auffenbergi* n. sp. from *L. smaragdina* collected in the Philippines are ovoidal, with a smooth, colourless, bi-layered wall, measure 19.9 × 15.8 μm, L/W 1.3; both micropyle and oöcyst residuum are absent, but one to four polar granules are present. Sporocysts are ovoidal to ellipsoidal, 10.3 × 5.8 μm, L/W 1.8, and the wall is composed of two valves joined by a longitudinal suture; neither Stieda nor sub-Stieda bodies are present; a sporocyst residuum is composed of dispersed granules.

Introduction

The emerald tree skink, *Lamprolepis smaragdina* (Lesson) is a fairly common and widespread arboreal lizard that occurs from the Wallacea islands, the Philippines, New Guinea, and Melanesia, to islands of the western Pacific (Perry & Buden, 1999; Linkem et al., 2012). It is one of the most common reptiles in villages, coconut plantations, secondary vegetation

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and gardens, where it occurs in foliage of bushes and on tree trunks (Foufopoulos & Richards, 2007). The emerald tree skink feeds mainly on beetles, larval lepidopterans, and fruit (Auffenberg & Auffenberg, 1988).

Although information is available on the ecology of *L. smaragdina* (see Auffenberg & Auffenberg, 1988; Perry & Buden, 1999; Linkem et al., 2012), little, to our knowledge, has been published on its parasites. For example, Austin & Perkins (2006) examined several *L. smaragdina* for hemoparasites, but did not find any, and Goldberg et al. (2010) examined 40 *L. smaragdina* from PNG and identified five nematode species (four as adult, one as encysted larval parasites), but no flatworm or acanthocephalan species. To date, there are no reports of coccidian parasites from this skink. Here, we document the sporulated oöcysts of two coccidian parasites, presumably *Eimeria* species (see Remarks sections), from emerald tree skinks collected from PNG and the Philippines.

Materials and methods

During June, 1991, one adult *L. smaragdina* was collected by hand from New Ireland, PNG, and in June, 2009, two adult *L. smaragdina* were collected by hand from Barangay Zabali, Campus of the Aurora State College of Technology (ASCOT), Luzon, Philippines. Fresh faecal samples were collected from each individual and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) (Gardner et al., 2012). They were later examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). All three samples contained unsporulated and partially sporulated oöcysts and they were placed in Petri dishes containing 2.5% $K_2Cr_2O_7$ and allowed to complete sporulation for five days at room temperature (*c.*23 °C). Still later, samples were again examined after flotation and measurements were taken on at least 25 oöcysts using a calibrated ocular micrometer or Olympus© cellSens 1.7 digital imaging software, and reported in micrometres, with means followed by the ranges in parentheses. Photographs were taken using Nomarski interference-contrast (DIC) optics. Oöcysts were between 300 (PNG) and *c.*1,095 (Philippines) days old when measured and photographed. Descriptions of the oöcysts and sporocysts follow the guidelines of Wilber et al. (1998). A symbiotype voucher specimen of *L. smaragdina* from PNG was accessioned into the Texas Natural History Collection (TNHC), Austin, Texas, USA, and those from the Philippines were accessioned into Natural History Museum, University of Kansas (KUMNH), Lawrence, Kansas, USA. Photosyntype vouchers of sporulated oöcysts were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA. Lizard taxonomy follows the TIGR reptile database (Uetz, 2013) and Zug (2013).

Results

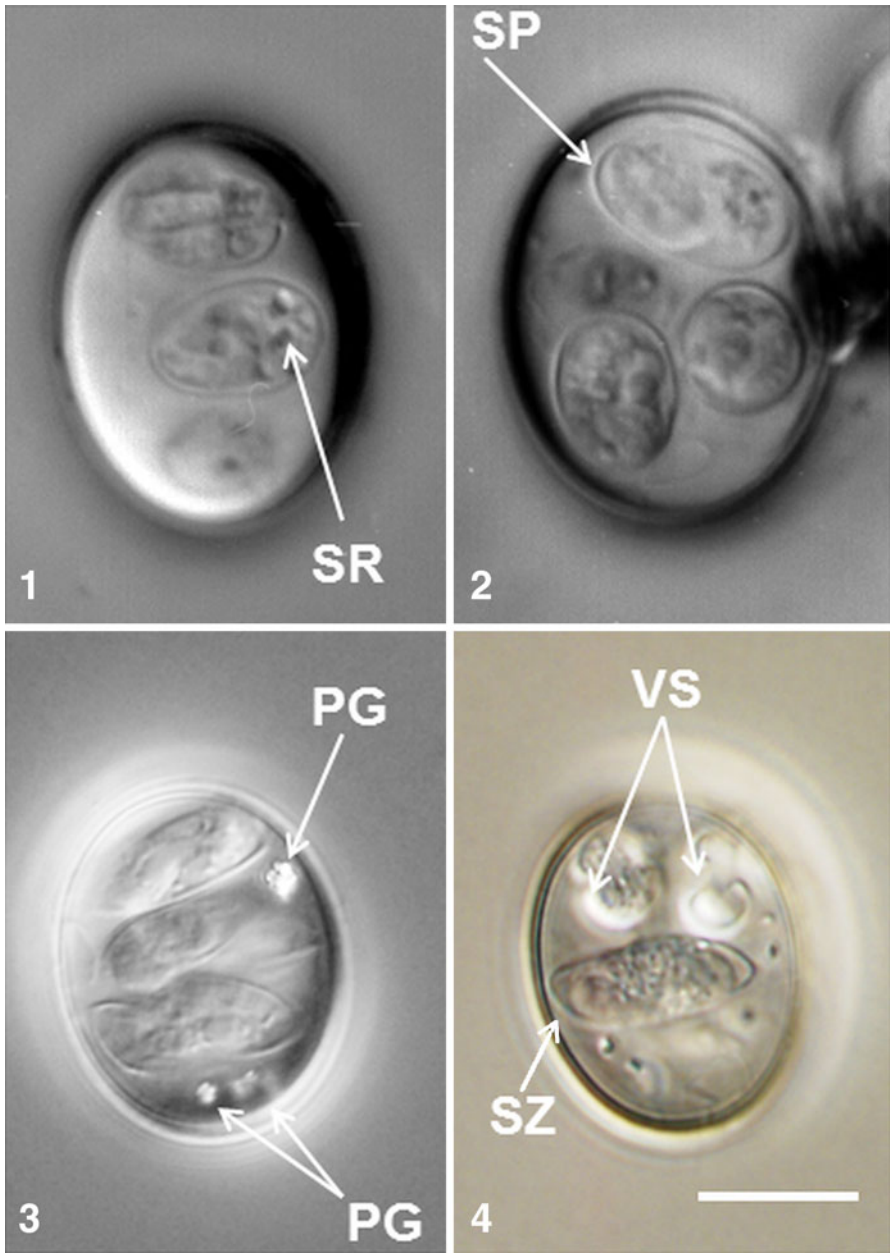
All three *L. smaragdina* from PNG and the Philippines were found to be passing oöcysts of what we believe are two undescribed coccidians; their descriptions follow.

Eimeria nuiailan n. sp.

Type-host: Emerald treeskink, *Lamprolepis smaragdina* (Lesson) (Sauria: Scincidae), symbiotype TNHC 51465 (field number CCA 754) collected June 1991.
Type-locality: Kavieng, New Ireland Province, PNG (2.566466°S, 150.798547°E), at sea level.
Type-material: Photosyntype (see Duszynski, 1999) as USNPC No. 106965.
Prevalence: 1/1 in the type-host.
Sporulation time: Unknown. Specimens were collected in the field, stored in $K_2Cr_2O_7$ solution, and not examined until 300 days later.
Site of infection: Unknown. Oöcysts were passed in faeces and host tissues were not collected or preserved for histological sectioning.
Etymology: The specific epithet is given for the Province of New Ireland in Tok Pisin, the official language of PNG. It is a form of Melanesian Pidgin English that was developed in the early 1800's.

Description (Figs. 1, 2, 5)

Sporulated oöcyst
Oöcyst (n = 25) colourless, smooth, ovoidal, 23.7 × 19.1 (21–26 × 17–22); length/width (L/W) ratio 1.3 (1.2–1.4). Wall bi-layered, *c.*1.2–1.4; outer layer *c.*0.8–1.0, inner layer *c.*0.4–0.6. Micropyle



Figs. 1–4 DIC photomicrographs of oöcysts of *Eimeria nuiailan* n. sp. (1–2) and *Eimeria auffenbergi* n. sp. (3–4). *Abbreviations*: PG, polar granule; SR, sporocyst residuum; SP, sporocyst; SZ, sporozoite; VS, valve suture. *Scale-bar*: 10 μm

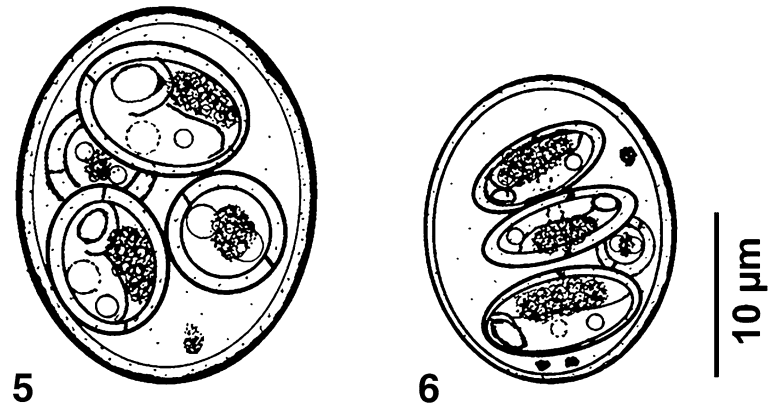
absent, oöcyst residuum absent; fragmented polar granule present.

Sporocyst

Sporocysts (n = 20) four, colourless, smooth, ovoidal to ellipsoidal, 11.9 × 7.0 (10–14 × 6–8); L/W ratio 1.7 (1.4–2.1); wall single-layered, *c*.0.6, composed of 2 valves, joined by a longitudinal suture. Stieda body, sub-Stieda body and para-Stieda body absent; sporocyst residuum (n = 10) spheroidal to subspheroidal, 4.4 × 3.2 (3–6 × 3–4) composed of compact mass of granules.

Sporozoite

Sporozoites (n = 10) two, sausage-shaped, 14.6 × 2.6 (13–17 × 2–3) *in situ*, lying along one side of



Figs. 5–6 Composite line drawings of oöcysts of *Eimeria nuiailan* n. sp. (5) *and Eimeria auffenbergi* n. sp. (6)

sporocyst; single ellipsoidal posterior refractile body (n = 5), 3.6 × 2.4 (3–5 × 2–3); spheroidal anterior refractile body (n = 5), 2.0 (1.6–2.2), with nucleus between them.

Remarks

Sporulated oöcysts of eimerians previously reported from skinks (see Modrý & Jirků, 2006) that are most similar to *E. nuiailan* are those of *Eimeria ablephari* Cannon, 1967, from the snake-eyed skink, *Cryptoblepharus* (=*Ablepharus*) *boutonii* (?) (species uncertain) from Australia with oöcysts that measured 23.1 × 17.7, with L/W ratio of 1.3 (Cannon, 1967); oöcysts of *Eimeria beyerae* Ovezmukhammedov, 1977 from the desert lidless skink, *Ablepharus deserti* from northern Turkmenistan (Ovezmukhammedov, 1977) that measured 23.1 × 16.2, with L/W ratio 1.4; and oöcysts of *Eimeria zugi* McAllister, Duszynski, Fisher & Austin, 2013 from *Carlia eothen* Zug and Admiralty brown skink, *Carlia ailanpalai* Zug, both from PNG, that measured 25.1 × 15.5, with L/W ratio 1.6 (McAllister et al., 2013c). However, we believe the oöcysts of *E. nuiailan* can be differentiated from the other three as follows: those of *E. nuiailan* possess a polar granule which both *E. ablephari* and *E. beyerae* lack, and sporocysts of *E. nuiailan* are much larger than those of both *E. ablephari* and *E. beyerae* (11.9 × 7.0 *vs* 8.9 × 6.6 and 9.4 × 6.6, respectively), with a larger L/W ratio (1.7 *vs* 1.3 and 1.4), respectively. Our new species has oöcysts that differ from those of *E. zugi* by having a much smaller oöcyst L/W ratio (1.3 *vs* 1.6).

Generic classification of the coccidia, historically, has been based on the structure of the sporulated oöcysts recovered from faecal material since this is the stage most readily available to investigators doing parasite survey work. The genus *Eimeria*, characterised by sporulated oöcysts with four sporocysts, each containing two sporozoites, has been a "catch-all" for such descriptions, but the tremendous diversity of such forms has prompted recent investigators to look more carefully at those coccidians parasitising reptiles. Paperna & Landsberg (1989) noted that certain *Eimeria*-like oöcysts had specific location and/or developmental patterns within their reptile hosts and erected two new genera: *Acroeimeria* Paperna & Landsberg, 1989 for those species that develop immediately beneath the brush-border of the intestinal epithelium and later have endogenous stages that extrude to form a layer on the surface of the gut mucosa, and *Choleoeimeria* Paperna & Landsberg, 1989 for those species that develop exclusively in the biliary tract of reptiles and almost always produce elongate-ellipsoidal oöcysts. Sporocysts of the species placed in both genera lack both Stieda and sub-Stieda bodies and usually are composed of two valves joined by a longitudinal suture presenting a "bi-valved" appearance. It is certainly possible that the morphotype we describe and name here as *E. nuiailan* n. sp. may be found by future investigators to have endogenous developmental characteristics to place it in the

Acroeimeria; however, since we were unable to examine intestinal tissues for endogenous stages nor do we have DNA sequences, we think it is prudent to name this form as a new eimerian until future studies can prove otherwise.

Eimeria auffenbergi n. sp.

Type-host: Emerald tree skink, *Lamprolepis smaragdina* (Sauria: Scincidae), Symbiotype KUMNH 323234, collected 3 June 1999.
Type-locality: Barangay Zabali, Campus of the Aurora State College of Technology (ASCOT), Luzon, Philippines (15°44.59'N, 121°34.54'E), elevation 50 m.
Type-material: Photosyntype (see Duszynski, 1999) as USNPC No. 106966.
Prevalence: 2/2 of the type-host.
Other host and locality: Another *L. smaragdina* (KUMNH 323232) collected on same date and locality as symbiotype.
Sporulation time: Unknown. Specimens were collected in the field, stored in $K_2Cr_2O_7$ solution, and not examined until 1,095 days later.
Site of infection: Unknown. Oöcysts were passed in the faeces and host tissues were not collected or preserved for histological sectioning.
Etymology: The specific epithet is given as a patronym in honor of the late Walter Auffenberg (1928–2004), University of Florida, for his numerous studies on the systematics and biology of reptiles, including skinks from the Philippines.

Description (Figs. 3–4, 6)

Sporulated oöcyst
Oöcyst (n = 31) colourless, smooth, ovoidal, 19.9 × 15.8 (17–22 × 14–18); length/width (L/W) ratio 1.3 (1.1–1.4). Wall bi-layered, *c*.0.8–1.2; outer layer, *c*.0.3–0.7, inner layer *c*.0.2–0.5. Micropyle absent, oöcyst residuum absent; polar granules (1–4) present.

Sporocyst
Sporocysts (n = 21) four, colourless, smooth, ellipsoidal, 10.3 × 5.8 (9–13 × 3–7); L/W ratio 1.8 (1.4–2.4); wall single-layered, *c*.0.5, composed of 2 valves joined by a longitudinal suture. Stieda body, sub-Stieda body and para-Stieda body absent; sporocyst residuum spheroidal to subspheroidal, composed of dispersed granules.

Sporozoite
Sporozoites (not measured) two, comma-shaped, lying along one side of sporocyst; single ellipsoidal posterior refractile body and spheroidal anterior refractile body, with nucleus between them.

Remarks

The following eimerians from skinks are most similar to *E. auffenbergi*: *Eimeria lampropholidis* Cannon, 1967 from the pale-flecked garden sunskink, *Lampropholis guichenoti* from Australia, and *Eimeria leiolopismatis* Cannon, 1967 from the orange-tailed shadeskink, *Saproscincus challengeri* from Australia (Cannon, 1967). However, sporulated oöcysts of *E. auffenbergi* can be differentiated from their oöcysts as follows: *E. auffenbergi* has polar granules, which *E. lampropholidis* lacks; the sporocysts of *E. auffenbergi* are larger, with a greater L/W ratio, than those of *E. lampropholidis* (10.3 × 5.8, L/W 1.8 *vs* 7.7 × 6.5, L/W 1.2, respectively), and they have smaller sporocysts, with a larger L/W ratio than those of *E. leiolopismatis* (10.3 × 5.8, L/W 1.8 *vs* 11.5 × 8.1, L/W 1.4, respectively).

There are at least two other factors we think merit consideration. First, the oöcysts available to us were >3 yrs old when examined and, in older sporocysts with sutures, it is not uncommon for the walls to disintegrate along the suture-lines, significantly changing their appearance. Thus, in some views of the oöcysts/sporocysts we had to examine, it appeared there may be a tiny Stieda body on the end of some sporocysts; however, we attribute those observations as light refraction of the collapsed sporocyst wall. Our second point is the possibility that the oöcysts we describe and name here as *E. auffenbergi* n. sp. may be found by future investigators to have endogenous developmental characteristics to place it in the genus *Acroeimeria* (*sensu* Paperna & Landsberg, 1989); however, since we were unable to examine intestinal tissues for endogenous stages and do not have DNA sequences, we think it is prudent to name this form as a new eimerian until empirical evidence is available to prove otherwise.

Discussion

For the last two decades we have been collecting skinks representing eight genera [*Carlia* (Gray), *Cryptoblepharus* Wiegmann, *Emoia* Gray, *Lipinia* Gray, *Lamprolepis* Fitzinger, *Papuascincus* Allison & Greer, *Prasinohaema* Greer, *Sphenomorphus* Fitzinger] from PNG and other South Pacific islands and examining their faeces for coccidia. These results are just now being published (McAllister et al., 2013a, b, c, 2014) and it appears that each skink species sampled has discharged oöcysts of at least one coccidian that is unique to it. Herein we have described two new species of *Eimeria* from a single, widely distributed host species (*L. smaragdina*). When compared to other genera examined above, these new eimerians were found only in emerald tree skinks, and each (*E. nuiailan* and *E. auffenbergi*) were also geographically restricted to PNG and the Philippines, respectively. We suggest that the host and geographic specificity of these parasites promises a wealth of undiscovered coccidian diversity that we are just now learning about.

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