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Four new species of coccidia (Apicomplexa: Eimeriidae) from Owen Stanley skinks, *Papuascincus stanleyanus* (Sauria: Scincidae), from Papua New Guinea

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Abstract: Between September and November 1991, 12 Owen Stanley skinks, *Papuascincus stanleyanus* (Boulenger) were collected from various localities on Papua New Guinea and examined for coccidians. Six (50%) were found to harbour four eimerians that we describe here as new. Oocysts of *Eimeria burseyi* sp. n. were elongate to ellipsoidal with a bilayered wall and measured (length × width, L × W) 36.0 × 24.0 µm, with a L/W ratio of 1.5. Both micropyle and oocyst residuum were absent, but a polar granule was present. Oocysts of *Eimeria goldbergi* sp. n. were ellipsoidal, with a bilayered wall, and measured 21.4 × 16.1 µm; L/W ratio was 1.3. Both micropyle and oocyst residuum were absent, but a single or fragmented polar granule was present. Oocysts of *Eimeria boulengeri* sp. n. were spheroidal to slightly subspheroidal, with a thin, single-layered wall that readily collapses, and measured 16.0 µm, L/W ratio was 1.0. Both micropyle and oocyst residuum were absent, but usually one (sometimes two) polar granule(s) were present. Oocysts of *Eimeria niuginiensis* sp. n. were oblong to tapered with a bilayered wall, and measured 20.0 × 13.1 µm; L/W ratio was 1.5. A micropyle, oocyst residuum and polar granule were absent. To our knowledge, these represent the only coccidians ever described from *P. stanleyanus*.

Keywords: Coccidia, *Eimeria*, taxonomy, oocysts, taxonomy, hosts, Reptilia

Skinks of the genus *Papuascincus* Allison et Greer, 1986 are small diurnal and terrestrial lizards endemic to the island of New Guinea and restricted to the upper montane region (>1000 m elevation and up to at least 3000 m) in the central highlands and north coast ranges (Allison 1982, 1996, Zweifel 1980, Allison and Greer 1986). Species of *Papuascincus* are distinguished from other lygosomine skinks by pustulate structures on the surface of their egg shell, an apomorphy unique amongst skinks and possibly amongst squamates (Allison and Greer 1986).

The affinities of *Papuascincus* appear to lie with three other New Guinean genera, *Lipinia* Gray, 1845, *Lobulia* Greer, 1974 and *Prasinohaema* Greer, 1974, all members of a tribal level informal grouping among lygosomine skinks, the *Sphenomorphus* group sensu Greer (1979). Presently four species names are applied within *Papuascincus*: *P. morokanus* (Parker), *P. stanleyanus* (Boulenger), *P. buergersi* (Vogt) and *P. phaedoes* (Vogt). The latter two taxa are known only from their holotypes, probably collected in the Sepik River Basin.

McAllister et al. (2013a) provided descriptions of two new isosporans from skinks of the genus *Emoia* Gray from Papua New Guinea (PNG) and the Fiji Islands. Their study represented the first report of coccidia from any reptile species from PNG. In addition, McAllister et al. (2013b–d, 2014) provided descriptions of new eimerians from *Carlia* Gray, *Lipinia*, *Lamprolepis* Fitzinger, and *Sphenomorphus* Fitzinger, skinks from the South Pacific, respectively. Although information is available on hemoparasites and helminths of Owen Stanley skinks (*P. stanleyanus*) from PNG (Austin and Perkins 2006, Bursey et al. 2008, 2010), nothing, to our knowledge, has been published on coccidia from this lizard. Herein, we provide descriptions of four new *Eimeria* spp. from *P. stanleyanus* from PNG.

MATERIALS AND METHODS

Between September and November 1991, 12 adult *Papuascincus stanleyanus* were collected by one of us (CCA) by hand or blowpipe from localities ranging from 1200–2000 m in Madang (n = 11) and Morobe (n = 1) provinces, PNG, and examined for

coccidians. Fresh faecal samples were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30).

Measurements were taken on 25 sporulated oocysts using a calibrated ocular micrometer and reported in micrometers (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were ~300 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 granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoite (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N).

Host vouchers were accessioned into the Texas Natural History Collection (TNHC), University of Texas, Austin, Texas, USA. Photosyntypes of sporulated oocysts were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA. Lizard taxonomy follows the reptile database (Uetz and Hošek 2013). Unfortunately, no host tissues were made available for us to study.

RESULTS

Eimeria burseyi sp. n.

Figs. 1, 2, 9

Description of sporulated oocyst: Oocyst with 4 sporocysts; shape elongate-ellipsoidal; bilayered wall, colourless, ~1.6 thick, smooth outer layer, inner and outer layers of equal thickness; $L \times W$: 36.0×24.0 (35–38 \times 22–26); L/W : 1.5 (1.4–1.6); M, OR, PG: all absent.

Description of sporocyst and sporozoites: SP ovoidal, with a smooth unilayered wall, ~0.4 thick, composed of 2 valves joined by a longitudinal suture; $L \times W$: 12.1×10.6 (12–13 \times 10–12); L/W : 1.1 (1.0–1.2); SB, SSB, PSB: all absent; SR: spheroidal, 6.0 (5–7), composed of moderately-sized globules in a compact mass or dispersed between SZ; SZ: sausage-shaped with spheroidal ARB and PRB; single N slightly posterior to midpoint of body.

Type host: *Papuascincus stanleyanus* (Boulenger) (Sauria: Scincidae). Collected 8 September 1991.

Type locality: Kaironk Village, 10 km NW of Simbai, Madang Province, Papua New Guinea (5°14'15"S; 144°28'50"E).

Other host species: None.

Prevalence: In one of 12 (8%) of the type host.

Site of infection: Unknown, oocysts recovered from faeces.

Materials deposited: Symbiotype host in the TNHC as No. 51767. Photosyntype of sporulated oocyst deposited in the USNPC as No. 106457.

Etymology: The specific epithet is given in honour of Dr. Charles R. Bursey, Professor of Biology, Pennsylvania State University-Shenango Campus, Sharon, Pennsylvania, USA, in recognition of his contributions to our knowledge of the helminth parasites of *P. stanleyanus*.

Remarks. We have placed this new coccidium tentatively in the genus *Eimeria* Schneider, 1875, because the sporulated oocysts are tetrasporocystic. However, we are fully aware that some tetrasporocystic eimeriid-like coccidians have been separated from *Eimeria* (such as *Choleoeimeria* Paperna et Landsberg, 1989, *Acroeimeria* Paperna et Landsberg, 1989), because they exhibit a suite of very specific characteristics in both endogenous and exogenous stages that differentiate them from members of *Eimeria* (e.g. sporulated oocysts significantly longer than wide, endogenous sporulation, sporocysts with wall sutures, endogenous development in gall bladder and bile duct epithelium, others; see Paperna and Landsberg 1989, Modrý and Jirků 2006, Sloboda and Modrý 2006). Although *E. burseyi* sporocysts seem to have a distinct suture, we have no other empirical evidence that our oocysts were shed completely sporulated or that the endogenous stages that produced these oocysts were located in the gall bladder/bile duct epithelium. Thus, we take the conservative path not to place this species into the genus *Choleoeimeria* until endogenous and/or molecular evidence is available to do so (or not).

In comparing sporulated oocysts (the only stage available to us) to those most similar in structure, those of *E. burseyi* are most similar to the oocysts of *Choleoeimeria saqanqouri* Abdel-Baki, El-Fayomi, Sakran et Abdel-Haleem, 2008, from the sandfish skink, *Scincus scincus* Linnaeus from Egypt (Abdel-Baki et al. 2008). However, the bilayered oocyst wall of *C. saqanqouri* measures 1.0 μm , whereas that of *E. burseyi* is thicker (1.6 μm) and the sporocyst width (and range) is larger in *E. burseyi* (10.6 [10–12] vs 8.9 [7.5–10] μm). We make this comparison because *E. burseyi* might be found later to have molecular or endogenous characters to allow its placement in the genus *Choleoeimeria* (see Discussion).

Eimeria goldbergi sp. n.

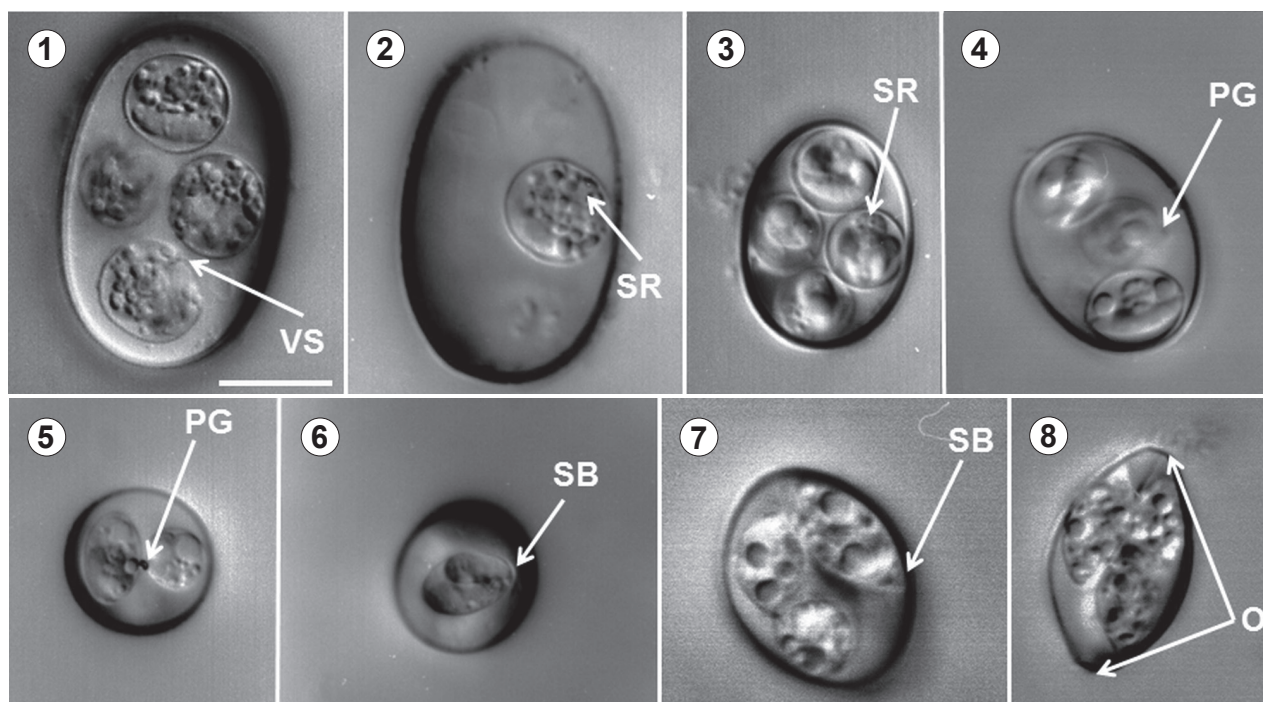
Figs. 3, 4, 10

Description of sporulated oocyst: Oocyst with 4 sporocysts; shape ellipsoidal; bilayered wall, colourless, ~1.4 thick, smooth outer layer ~0.8–1.0, inner layer ~0.4–0.6; $L \times W$: 21.4×16.1 (20–23 \times 15–19); L/W : 1.3 (1.2–1.5); M, OR: both absent; PG: single or often fragmented.

Description of sporocyst and sporozoites: SP ovoidal, with a smooth unilayered wall, ~0.4 thick, composed of 2 valves joined by a longitudinal suture; $L \times W$: 9.4×6.9 (8–10 \times 6–8); L/W : 1.4 (1.2–1.6); SB, SSB, PSB: all absent; SR: spheroidal, 3.0 (2.6–3.2), composed of moderately sized globules dispersed between SZ; SZ: sausage-shaped, 2.5 wide (2–3), with spheroidal ARB 1.9 (1.6–2.4) and spheroidal PRB 2.5 (2.2–2.6); single N slightly posterior to midpoint of body.

Type host: *Papuascincus stanleyanus* (Boulenger) (Sauria: Scincidae). Collected 8 September 1991.

Type locality: Kaironk Village, 10 km NW Simbai, Madang Province, Papua New Guinea (5°14'15"S; 144°28'50"E).



Figs. 1–8. Oocysts of new species of *Eimeria* from *Papuascincus stanleyanus*, Nomarski interference-contrast photomicrographs. **Figs. 1, 2.** *Eimeria burseyi* sp. n. **Figs. 3, 4.** *Eimeria goldbergi* sp. n. **Figs. 5, 6.** *Eimeria boulengeri* sp. n. **Figs. 7, 8.** *Eimeria niuginiensis* sp. n. Abbreviations: O – oocyst; PG – polar granule; SB – Stieda body; SR – sporocyst residuum; VS – valve suture. Scale bar = 10 µm for all figures.

Other host species: None.

Prevalence: In three of 12 (25%) of the type host.

Site of infection: Unknown, oocysts recovered from faeces.

Materials deposited: Symbiotype host in the TNHC as No. 51772. Photosyntype of sporulated oocyst deposited in the USNPC as No. 106458.

Etymology: The specific epithet is given in honour of Stephen R. Goldberg, Professor of Biology, Whittier College, Whittier, California, USA, in recognition of his contributions to our knowledge of the helminth parasites of *P. stanleyanus*.

Remarks. Similar to the reasoning and argument presented above for *E. burseyi*, we also have placed this new coccidium, tentatively, into the genus *Eimeria* because the sporulated oocysts are tetrasporocystic. Sporulated oocysts of *E. goldbergi* are most similar in size to two other *Eimeria*-like coccidians from skinks with intracytoplasmic development, but considered by Modrý and Jirků (2006) to be *insertae sedis* as follows: *Eimeria ablephari* Cannon, 1967 from the snake-eyed skink, *Cryptoblepharus boutonii* (Des Jardin) from Australia has oocysts that measure $23.1 \times 17.7 \mu\text{m}$ ($L/W = 1.3$) and SP that measure $8.9 \times 6.6 \mu\text{m}$ ($L/W = 1.3$) (Cannon 1967) and *Eimeria maboia* Carini, 1938, from the Paraguay mabuya, *Aspronema dorsivittata* (Cope) and the greater Martinique skink, *Mabuya mabouia* (Bonnaterre) from Brazil (Carini 1938) that possesses oocysts that measure $20 \times 17 \mu\text{m}$ ($L/W = 1.2$) and SP that measure $9 \times$

7.5 ($L/W = 1.2$). However, *E. goldbergi* possesses a PG, while *E. ablephari* and *E. maboia* do not.

***Eimeria boulengeri* sp. n.**

Figs. 5, 6, 11

Description of sporulated oocyst: Oocyst with 4 sporocysts; oocyst shape spheroidal to slightly subspheroidal; thin, colourless, unilayered wall, ~ 0.8 thick (readily collapses); $L \times W$: 16.0 ($14\text{--}18$); L/W : 1.0 ($1.0\text{--}1.1$); M, OR: both absent; PG: usually 1, sometimes 2, ~ 2.0 wide.

Description of sporocyst and sporozoites: SP ovoidal, with a smooth, thin, colourless unilayered wall; $L \times W$: 9.8×6.5 ($9\text{--}11 \times 6\text{--}10$); L/W : 1.5 ($1.1\text{--}1.8$); SB: very small; SSB, PSB: both absent; SR: spheroidal, 2.8 ($2\text{--}3$), composed of moderately-sized globules dispersed between SZ; SZ: sausage-shaped with spheroidal ARB 1.9 ($1\text{--}3$) and ovoidal PRB 3.6×2.7 ($2\text{--}6 \times 2\text{--}3$); single N slightly posterior to midpoint of body.

Type host: *Papuascincus stanleyanus* (Boulenger) (Sauria: Scincidae). Collected 8 September 1991.

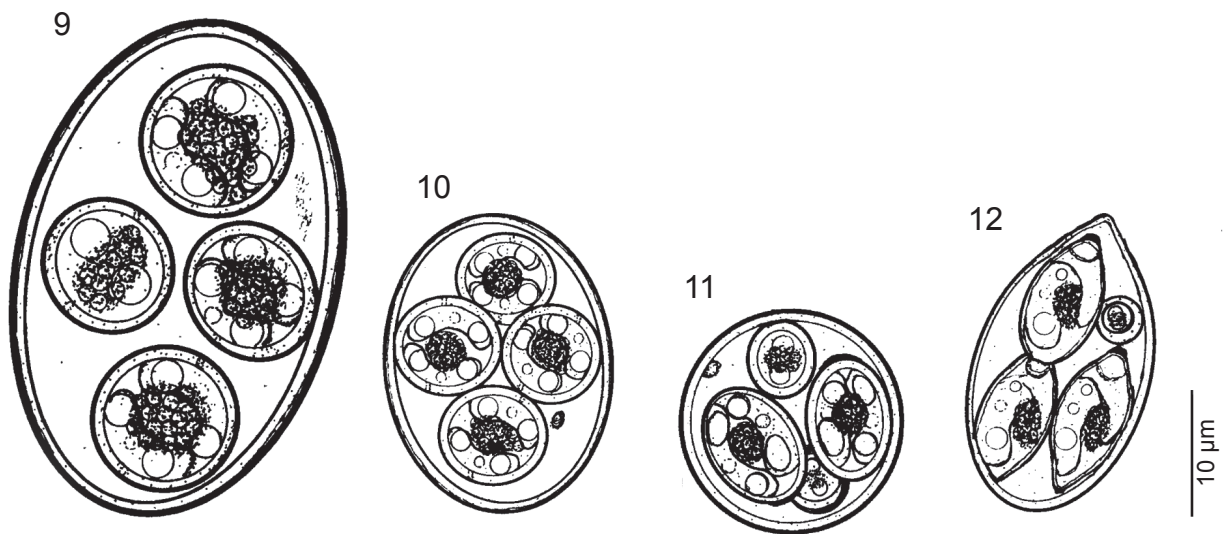
Type locality: Kaironk Village, 10 km NW Simbai, Madang Province, Papua New Guinea ($5^{\circ}14'15''\text{S}$; $144^{\circ}28'50''\text{E}$).

Other host species: None.

Prevalence: In one of 12 (8%) of the type host.

Site of infection: Unknown, oocysts recovered from faeces.

Materials deposited: Symbiotype host in the TNHC as No. 51776. Photosyntype of sporulated oocyst deposited in the USNPC as No. 106459.



Figs. 9–12. Oocysts of new species of eimeria from *Papuascincus stanleyanus*; composite line drawings of. **Fig. 9.** *Eimeria burseyi* sp. n. **Fig. 10.** *Eimeria goldbergi* sp. n. **Fig. 11.** *Eimeria boulengeri* sp. n. **Fig. 12.** *Eimeria niuginiensis* sp. n.

E t y m o l o g y : The specific epithet is given in honour of Belgian taxonomist Dr. George Albert Boulenger (1858–1937) who described the type host in 1897.

Remarks. There is a single eimerian with spheroidal oocysts from skinks, *Eimeria sami* Bovee, 1971, from the Ousima skink, *Plestiodon marginatus* Hallowell, 1861, from Japan (Bovee 1971). Oocysts of *E. sami* are 18 (15–20) μm wide with ellipsoidal SP measuring $9 \times 7.5 \mu\text{m}$ with a L/W ratio of 1.2. However, our new species differs from *E. sami* by having 1–2 polar granule(s), which are not present in *E. sami*, as well as by having a considerably larger SP L/W ratio (1.5).

***Eimeria niuginiensis* sp. n.**

Figs. 7, 8, 12

Description of sporulated oocyst: Oocyst with 4 sporocysts; oocyst shape oblong and tapered at one end; colourless, bilayered wall, ~ 1.0 thick, smooth, outer and inner layers of equal thickness; $L \times W$: 20.0×13.1 (18–22 \times 12–14); L/W: 1.5 (1.3–1.7); M, OR, PG: all absent.

Description of sporocyst and sporozoites: SP ovoidal; smooth, colourless, unilayered wall ~ 0.3 thick; $L \times W$: 12.4×5.8 (12–13 \times 5–6); L/W: 2.1 (1.9–2.5); SB, SSB: both present; PSB: absent; SR: present as scattered granules among SZ; SZ: sausage-shaped, 2.3 wide (2.2–2.4) with spheroidal ARB 1.3 (1.1–1.6) and spheroidal PRB, 3.2×2.1 (3.0–3.2 \times 2.0–2.2); single N slightly posterior to midpoint of body.

Type host: *Papuascincus stanleyanus* (Boulenger) (Sauria: Scincidae). Collected 8 September 1991.

Type locality: Kaironk Village, 10 km NW Simbai, Madang Province, Papua New Guinea ($5^{\circ}14'15''\text{S}$; $144^{\circ}28'50''\text{E}$).

Other host species: None.

Prevalence: In one of 12 (8%) of the type host.

Site of infection: Unknown, oocysts recovered from faeces.

Materials deposited: Symbiotype host in the TNHC as No. 51776. Photosytype of sporulated oocyst deposited in the USNPC as No. 106460.

E t y m o l o g y : The specific epithet is given for the country where hosts were collected, the Independent State of Papua New Guinea, utilising the pidgin spelling Niugini.

Remarks. No eimerians were previously described from members of the family Scincidae (Modrý and Jirků 2006; Abdel-Baki et al. 2008, 2013, McAllister et al. 2013b,c,d, 2014) that have oocysts in any way similar to the distinctly shaped oblong and tapered oocysts of *E. niuginiensis*.

DISCUSSION

As we noted above, some tetrasporocystic eimeriid-like coccidians now have been separated from the genus *Eimeria*. For example, Paperna and Landsberg (1989) proposed *Choleoeimeria* as a new genus for the eimeriid-like coccidia with endogenous stages infecting the gall bladder epithelium of reptiles; they further characterised their new genus by long ellipsoidal oocysts (L/W ratio > 1.6), sporocysts without a SB, and sporulation that begins in the lumen of the gallbladder.

A phylogenetic analysis (SSU rDNA) by Jirků et al. (2002) gave some evidence to support the separate status of *Choleoeimeria* as a sister clade to *Eimeria* within the Eimeriidae. More recent morphological and molecular studies now lend increasing support for this concept (Modrý and Jirků 2006, Sloboda and Modrý 2006, McAllister 2012a,b). Therefore, the question can be asked, why did we not include *E. burseyi* and, perhaps, *E. goldbergi* in the genus *Choleoeimeria* since both have sporocysts with characteristic sutures and lacking a SB? The most parsimonious reason at this time is that we have no definitive, empirical proof that our oocysts were shed completely

sporulated, or that the endogenous stages that produced these oocysts were located in the gall bladder epithelium, because tissues were neither examined in the field nor fixed and collected for later examination. Finally, we did not sequence oocysts and have no other line of evidence to separate these oocysts from those of *Eimeria*. Thus, we choose to be conservative herein and although *E. burseyi* and *E. goldbergi* sporocysts possess sutures in their sporocyst walls, which could place them within *Choleoeimeria*, we do not have the necessary data to help us support our distinction to place them there.

Modrý and Jirků (2006) provided a summary and revision of *Eimeria*-like coccidia of the family Scincidae that included descriptions of new species of *Acroeimeria* and *Choleoeimeria* from *Marmorosphax tricolor* (Bavay) from New Caledonia. Since then, we are aware of four additional *Choleoeimeria* species described from skinks

(Abdel-Baki et al. 2008, 2013, Alyousif and Al-Shawa 2010, Al-Quraishy 2011). We provide descriptions of the first coccidians ever reported from skinks of the genus *Papuascincus* and the sixth (McAllister et al. 2013a–d, 2014) report of reptilian coccidia from PNG.

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