

Description of *Choleoimeria duszynskii* n. sp. (Apicomplexa: Eimeriidae) from the gallbladder of the Middle Eastern short-fingered gecko *Stenodactylus doriae* (Blanford) (Sauria: Gekkonidae) in Saudi Arabia

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Abstract *Choleoimeria duszynskii* n. sp. is described from the gallbladder of the Middle Eastern short-fingered gecko *Stenodactylus doriae* (Blanford) from Salasel, Central region, Saudi Arabia. Oöcysts are ellipsoidal (rarely ovoidal), $23\text{--}25 \times 16\text{--}18$ (24×17) μm , with mean length/width ratio 1.4. Oöcyst wall is smooth, bi-layered, *c.*1.0 μm thick. Micropyle, oöcyst residuum and polar granule are absent. Sporocysts are ellipsoidal, $8\text{--}10 \times 4\text{--}6$ (9×5) μm , with a smooth, colourless sporocyst wall and mean length/width ratio 1.7. Sporozoites are sausage-shaped, with one end slightly pointed, arranged head to tail around sporocyst residuum. Refractile bodies and nuclei are not discernible. The endogenous development is restricted to the epithelium of the gallbladder and bile duct. Meronts are rounded, 12 μm in diameter, containing up to *c.*15 merozoites. Microgamonts are irregular in shape, 22×17 μm , containing a large number of microgametes. Macrogamonts are spherical, 17 μm in diameter,

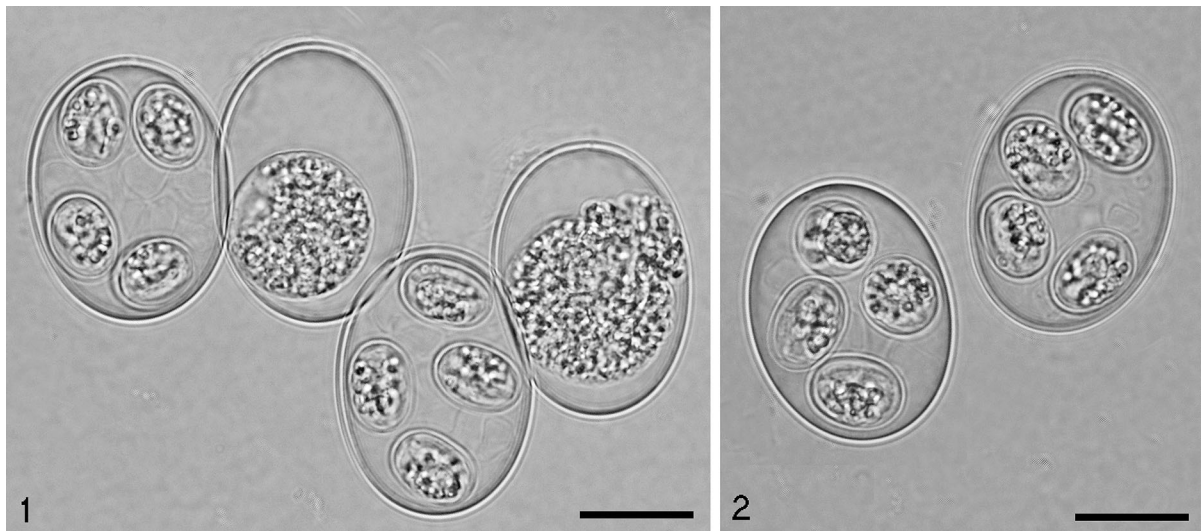
with centrally located nucleus and wall-forming bodies at the periphery.

Introduction

The infraorder Gekkota Cuvier is the most diverse lineage of lizards, represented by *c.*1,461 species in seven families, of which the Gekkonidae Gray is the largest with 942 nominal species (Uetz & Hošek, 2013). Of these, more than 48 species have been recorded in Saudi Arabia (Al-Sadoon, 2004). Within the Gekkonidae, the genus *Stenodactylus* Fitzinger is represented by 11 species; of these seven species are known from Saudi Arabia (Uetz & Hošek, 2013). *Stenodactylus doriae* (Blanford) is a typical psammophilous species inhabiting soft sand dunes in the Arabian Peninsula (Al-Oran, 2000). It occurs in Saudi Arabia, Iran, Iraq, Israel, the United Arab Emirates, Oman, Jordan, and Kuwait (Al-Sadoon, 2004). This species reaches about 8.3 cm in snout-to-vent length (Uetz & Hošek, 2013). Although no coccidia are known from *S. doriae*, *Eimeria stenodactyli* El-Toukhy, 1994 and *Isospora stenodactyli* El-Toukhy, 1994 have been described from *Stenodactylus sthenodactylus* (Lichtenstein) in Egypt (El-Toukhy, 1994a, b). To the best of our knowledge, no other records of coccidia from this host genus exist. In this paper we provide the description of a new species of *Choleoimeria* Paperna & Landsberg, 1989 from *S. doriae* in Saudi Arabia.

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Figs. 1–2 Photomicrographs of freshly collected sporulated oocysts of *Choleoeimeria duszynskii* n. sp. from the gallbladder of *Stenodactylus doriae*. Scale-bars: 10 μ m

Materials and methods

Sixteen adult specimens of the Middle Eastern short-fingered gecko *Stenodactylus doriae* (Blanford) were collected by hand from Salasel City (26°4'30"N, 49°27'13"E) in the Central region of Saudi Arabia during April 2013. Geckos were caged separately and infection was detected by demonstration of oocysts in the faeces. Positive geckos were then killed with chloroform and the infection in the gallbladder was verified by microscopic examination of the bile. Samples of the gallbladder together with portions of the liver, spleen, kidney, lung, and intestine were fixed in 10% neutral buffered formalin. The fixed tissues were then processed for histological examination, sectioned and stained with hematoxylin and eosin. All developmental stages were observed and photographed using an Olympus BX51 microscope with an Olympus DP71 camera (Olympus, Shinjuku, Tokyo, Japan). Measurements were made using ocular micrometer with an oil-immersion lens and the data are given in micrometres with ranges followed by the means \pm standard deviations in parentheses.

Results

During corpological examination of *S. doriae*, the presence of oocysts of the genus *Choleoeimeria* was

detected in two out of 16 geckos (12%). All oocysts studied exhibited similar morphological characteristics and we believe they represent a previously unreported species, which is described below.

Choleoeimeria duszynskii n. sp.

Type-host: Middle Eastern short-fingered gecko *Stenodactylus doriae* Blanford, 1874 (Sauria: Gekkonidae).

Type-locality: Salasel City (26°4'30"N, 49°27'13"E), in the central region of Saudi Arabia.

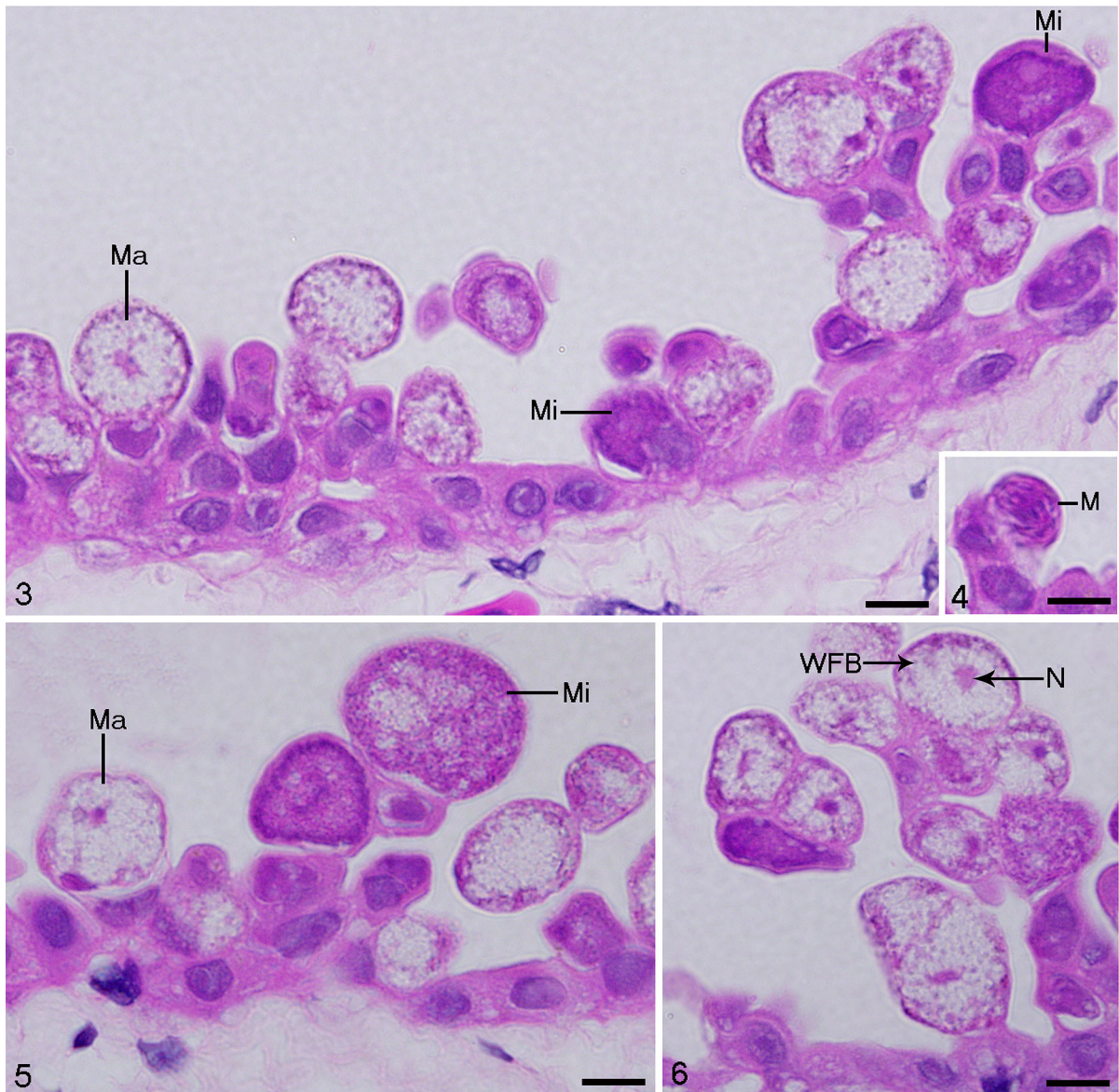
Prevalence: 12% (2 infected out of 16 geckos examined).

Site of infection: Gallbladder.

Sporulation: Endogenous; both sporulated and unsporulated oocysts were found in the lumen of the gallbladder and intestinal contents prior to being voided in the faeces.

Type-material: Photosyntype for the sporulated oocysts and one slide with syntypes of hematoxylin and eosin stained endogenous stages in the gallbladder epithelia are deposited in the Parasitological Collection of the Hungarian Natural History Museum (HNHM 70398).

Etymology: The specific epithet is given in honour of Professor Donald W. Duszynski, University of New Mexico, USA, for his great contribution in the field of coccidia.



Figs. 3–6 Photomicrographs of endogenous stages of *Choleoieimeria duszynskii* n. sp. in the gallbladder epithelia of *Stenodactylus doriae* showing mature meront with merozoites (M), microgamonts (Mi), macrogamonts (Ma) with wall-forming bodies arranged at the periphery (WFB) and a centrally located nucleus (N). Scale-bars: 10 μ m

Description (Figs. 1–7)

Exogenous stages

Oöcysts ellipsoidal (rarely ovoidal), 23–25 \times 16–18 (24 \pm 0.2 \times 17 \pm 0.4), length/width (L/W) ratio 1.3–1.5 (1.4) (Figs. 1, 2, 7). Oöcyst wall smooth, bi-layered, c.1.0 thick. Micropyle, oöcyst residuum and

polar granule absent. Sporocysts dizoic, ellipsoidal, 8–10 \times 4–6 (9 \pm 0.4 \times 5 \pm 0.3): sporocyst wall smooth, colourless, composed of 2 plates connected with meridian suture; sporocyst length/width (L/W) ratio 1.6–1.8 (1.7). Stieda body and sub-Stieda body both absent. Sporocyst residuum present as cluster of fine granules. Sporozoites sausage-shaped, with one

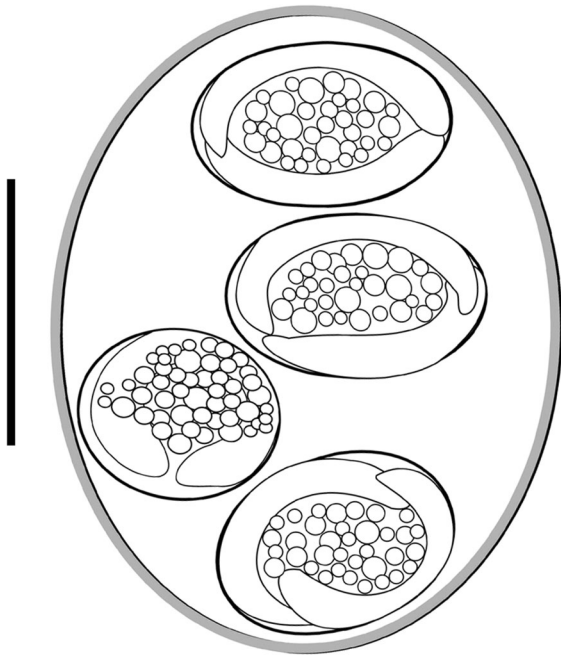


Fig. 7 Oocyst of *Choleoeimeria duszynskii* n. sp. from *Stenodactylus doriae*. Scale-bar: 10 μ m

end slightly more pointed, arranged head-to-tail within sporocysts; refractile bodies and nuclei not discernible.

Endogenous stages

The endogenous development takes place within the epithelial cells of the gallbladder and along the biliary duct, where it causes extensive destructions of the cells (Fig. 3). The infected epithelial cells become atrophied and displaced into the lumen of the gallbladder and remain in contact with the basal layer by a stalk-like structure (Fig. 3). No endogenous stages were found in any other organ examined. Meronts rounded, 11–14 (12) in diameter, containing up to *c.*15–16 merozoites (Fig. 4). Microgamonts irregular in shape, 18–24 \times 16–20 (22 \times 17) containing a large number of microgametes. Macrogamonts mostly spheroidal, 15–20 (17) wide, with centrally-located nucleus (Figs. 3, 5, 6); wall-forming bodies present in the periphery of macrogamont cytoplasm (Fig. 3).

Discussion

Eimeriid coccidia of reptiles represent a diverse assemblage of species differing in both exogenous

stages (oocysts) and endogenous development (Modrý et al., 2004). Based on the combination of the oocyst morphology, the site and characters of the endogenous development, Paperna & Landsberg (1989) suggested *Choleoeimeria* as a new genus to accommodate some tetrasporocystic *Eimeria*-like coccidia, infecting gallbladders of reptiles. The genus *Choleoeimeria* is characterised by elongate to ellipsoidal oocysts with sporulation in the lumen of the gallbladder, which lack Siteda body and undergo endogenous development in the gallbladder epithelium (Paperna & Landsberg, 1989). The separate generic status of *Choleoeimeria* has not received wide acceptance until its distinctiveness from *Eimeria* spp. has been confirmed by the molecular study of Jirků et al. (2002). Abdel-Baki et al. (2013) stressed the necessity of studying the endogenous stages of eimeriid coccidia as a fundamental tool in allocating them to a particular genus, especially when working with eimeriid coccidians discovered in cold blooded vertebrates. Based on the oocyst morphometry and the endogenous development, we assigned the present biliary coccidium as a new species of the genus *Choleoeimeria*.

To date, seven species of *Choleoeimeria* have been described from hosts of the family Gekkonidae (Table 1): *C. bunopusi* Al-Quraishy, Abdel-Baki & Al Otaibi, 2013; *C. carini* (Carini & Pinto, 1926); *C. flaviviridis* (Setna & Bana, 1935); *C. heteronotis* Paperna, 2007; *C. pachyductyli* Paperna & Landsberg, 1989; *C. turcicus* (Upton, McAllister & Freed, 1988) and *C. xiangmaii* Paperna, 2007 (see Carini & Pinto, 1926; Setna & Bana, 1935; Upton, McAllister & Freed, 1988; Paperna & Landsberg, 1989; Lainson & Paperna, 1999; Paperna, 2007; Al-Quraishy et al., 2013).

These species differ from *C. duszynskii* n. sp. as follows. The oocysts of *C. bunopusi* are longer and wider (30–33 \times 20–22 vs 23–25 \times 16–18 μ m), with longer sporocysts (11–13 vs 8–10 μ m). *Choleoeimeria carini* has longer, sometimes curved oocysts, with wider sporocysts and several small granules which exhibit Brownian movement. *Choleoeimeria flaviviridis* has longer and thinner oocysts with a larger L/W ratio (1.8–2.4 vs 1.3–1.5) and ovoidal sporocysts. Similarly, the oocysts of *C. pachyductyli* are longer than those of *C. duszynskii* n. sp., with a small knob at one pole of the oocyst, and a larger L/W ratio (2.05 vs 1.70). *Choleoeimeria turcicus* has longer cylindrical oocysts with a much larger L/W ratio (1.9–2.3 vs

Table 1 Comparative data for *Choleoeimeria duszynskii* n. sp. and morphologically similar species from hosts of the family Gekkonidae

Species	Host	Oöcyst size	Oöcyst shape (L/W ratio) ^a	Sporocyst size	Sporocyst shape (L/W ratio)	References
<i>Choleoeimeria bunopusi</i> Al-Quraishy, Abdel-Baki & Al Otaibi, 2013	<i>Bunopus tuberculatus</i> Blanford	30–33 × 20–22 (31 × 21)	Ellipsoidal 1.4–1.6 (1.5)	11–13 × 6–8 (12 × 7)	Ellipsoidal 1.2–1.5 (1.3)	Al-Quraishy et al. (2013)
<i>Choleoeimeria carini</i> (Carini & Pinto, 1926)	<i>Hemidactylus mabouia</i> Fitzinger	30–35 × 16–19 (32 × 18)	Cylindrical 1.6–2.0 (1.8)	10–11 × 8–9 (11 × 9)	Ellipsoidal 1–2 (1.2)	Lainson & Paperna (1999)
<i>Choleoeimeria duszynskii</i> n. sp.	<i>Stenodactylus doriae</i> (Blanford)	23–25 × 16–18 (24 × 17)	Ellipsoidal 1.3–1.5 (1.4)	8–10 × 4–6 (9 × 5)	Ellipsoidal 1.6–1.8 (1.7)	Present study
<i>Choleoeimeria flaviviridis</i> (Setna & Bana, 1935)	<i>Hemidactylus flaviviridis</i> Rüppell	25–34 × 11–14	Ellipsoidal 1.8–2.4	7–9 × 5–7	Ovoidal?	Paperna & Landsberg (1989)
<i>Choleoeimeria heteronotis</i> Paperna, 2007	<i>Heteronotia binoei</i> (Gray)	32–34 × 16–18 (33 × 17)	Ellipsoidal 1.8–2.1 (1.9)	9–10 × 6–8 (10 × 8)	Ellipsoidal 1.1–1.6 (1.3)	Paperna (2007)
<i>Choleoeimeria pachydactyli</i> Paperna & Landsberg, 1989	<i>Pachydactylus capensis</i> (Smith)	25–31 × 11–17 (28 × 14)	Cylindroidal 2.05	10–13 × 6–7 (11 × 7)	?	Paperna & Landsberg (1989)
<i>Choleoeimeria turcicus</i> (Upton, McAllister & Freed, 1988)	<i>Hemidactylus turcicus</i> (Linnaeus)	35–41 × 17–20 (38 × 18)	Cylindroidal 1.9–2.3 (2.1)	10–12 × 8–9 (11 × 9)	Ovoidal 1.1–1.4 (1.3)	Paperna & Landsberg (1989)
<i>Choleoeimeria xiangmaii</i> Paperna, 2007	<i>Chemidactylus frenatus</i> Schlegel in Dumeril & Birbon	29–33 × 13–16 (30 × 15)	Ellipsoidal 1.7–2.6 (1.9)	8–10 × 5–8 (9 × 6)	Ellipsoidal - Subspherical?	Paperna (2007)
<i>Eimeria stenodactyli</i> El-Toukhy, 1994	<i>Stenodactylus sthenodactylus</i> (Lichtenstein)	26–32 × 22–27 (28 × 24)	Oval - Subspherical? ^b	9–11 × 8–9 (10 × 8)	Ovoidal?	El-Toukhy (1994a)

^a L/W ratio, length/width ratio; ^b?, No data available

1.3–1.5) and one, or sometimes two, polar granules. In addition, this species has ovoidal sporocysts with a smaller L/W ratio (1.1–1.4 vs 1.6–1.8). Finally, both *C. xiangmaii* and *C. heteronotis* have longer, ellipsoidal and bent oöcysts with larger L/W ratios (1.7–2.6 and 1.8–2.1, respectively vs 1.3–1.5).

The L/W ratios of previously described species of *Choleoeimeria* and the new species described above deserve a comment since all forms compared with *C. duszynskii* n. sp. have much greater L/W ratios. However, in their original generic definition of the genus *Choleoeimeria*, Paperna & Landsberg (1989) stated: “Oöcysts cylindroid to oval with length/width

ratio >1.4 (usually 1.6–2.2)”. The oöcysts of the new species fall within the lower limit of the L/W ratio range, being ovoidal (vs cylindroidal) and with a L/W ratio 1.4. This helps to distinguish *C. duszynskii* n. sp. even more precisely from the previously described congeners.

Eimeria stenodactyli El-Toukhy, 1994 and *Isospora stenodactyli* El-Toukhy, 1994 are the only coccidia described from geckos of the genus *Stenodactylus* (see El-Toukhy, 1994a, b). *Eimeria stenodactyli* differs from the new species described above in having larger oöcysts, ovoidal sporocysts with Stieda and sub-Stieda bodies and endogenous development confined to the intestinal epithelium (Table 1).

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