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Choleoeimeria salaselensis sp. n. (Apicomplexa: Eimeriidae) from the gall bladder of the horned viper *Cerastes gasperettii* (Serpentes: Viperidae) in Saudi Arabia

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Abstract: Oocyst morphology and endogenous developmental stages are described for *Choleoeimeria salaselensis* sp. n. from the gall bladder of 10 horned vipers, *Cerastes gasperettii* Leviton and Anderson, in Saudi Arabia. Sporulated oocysts are ellipsoidal, 23 × 15 (22–25 × 14–17) µm, length/width ratio (L/W) 1.5 (1.4–1.6), each with 4 sporocysts (*Eimeria*-like), but lack a micropyle, polar granules and oocysts residuum. Sporocysts are ellipsoidal, 8 × 5 (7–9 × 5–6) µm, L/W 1.5 (1.4–1.6), and Stieda, substieda and parasubstieda bodies are all absent, but a longitudinal suture, which divided the sporocysts into 2 plates, is present. Endogenous development is confined to epithelial cells in the bile duct and gall bladder; mature meronts were 11 × 7 µm, each with 10–16 merozoites, microgamonts were ~12 µm wide, and macrogamonts were ~16 µm wide with a prominent nucleus and wall-forming bodies. Given these two diagnostic features, sporocysts with a suture and composed of two plates and endogenous development limited to the biliary epithelium, we believe this coccidium is best classified as a member of *Choleoeimeria* Paperna et Landsberg, 1989. There are 5 known *Eimeria* species from vipers that have sporocysts somewhat similar in size to those of our new form, but all of them have much larger oocysts and larger sporocysts, some of which differ significantly in shape; there are not yet any *Choleoeimeria* species known from the Viperidae.

Keywords: Coccidia, oocysts, endogenous stages, taxonomy, snakes

The genus *Eimeria* Schneider, 1875 is the largest apicomplexan genus and may be one of the most speciouse genera of all eukaryotes. The identification of most coccidian genera and species is traditionally based on the morphological characteristics exhibited by oocysts and sporocysts. For example, about 98% of the species in the genus *Eimeria* are known only from their sporulated oocyst, which has a limited number of structural characters. Sometimes the dimensions of these structures overlap among species and may vary within a single species (Gardner and Duszynski 1990, Upton et al. 1992).

Consequently, it is always advantageous to observe as many additional features as possible, such as the endogenous developmental stages (e.g. merogony, gamogony), to make more confident and reliable identifications, especially when examining reptile hosts for coccidians (Lainson and Paperna 1999, Modrý and Jirků 2006).

In 1989, Paperna and Landsberg proposed the genus *Choleoeimeria* to comprise some *Eimeria* and *Eimeria*-like coccidians that undergo their endogenous development in the biliary epithelium of reptiles. The separate status of *Choleoeimeria* as a sister clade to *Eimeria* was supported by the phylogenetic analysis (SSU rDNA) undertaken by Jirků et al. (2002) and, since then, has gained wide acceptance by many authors (e.g. Modrý and Jirků 2006, Sloboda and Modrý 2006, Abdel-Baki et al. 2008, 2009, 2013, Al-Quraishy 2011, McAllister 2012a,b). This stresses the necessity of revising the taxonomic status of *Eimeria*-like species from other lizards through study of the endogenous stages. Using this perspective, here we describe a new species of *Choleoeimeria* based on the exogenous and endogenous stages from the gall bladder of the horned viper, *Cerastes gasperettii* Leviton et Anderson, collected from Saudi Arabia.

MATERIALS AND METHODS

Ten adult specimens of the horned viper, *Cerastes gasperettii*, were captured from Salasel (26°4'30"N; 49°27'13"E), Central Region, Saudi Arabia, during a survey of coccidia in snakes in April 2013. The animals were caged separately and their infection was detected by demonstration of oocysts in their faeces. Three heavily infected snakes were then killed with chloroform and infection in the gall bladder was verified by microscopic examination of bile collected by puncturing the gall bladder with a finely pointed glass pipette. For histology, selected tis-

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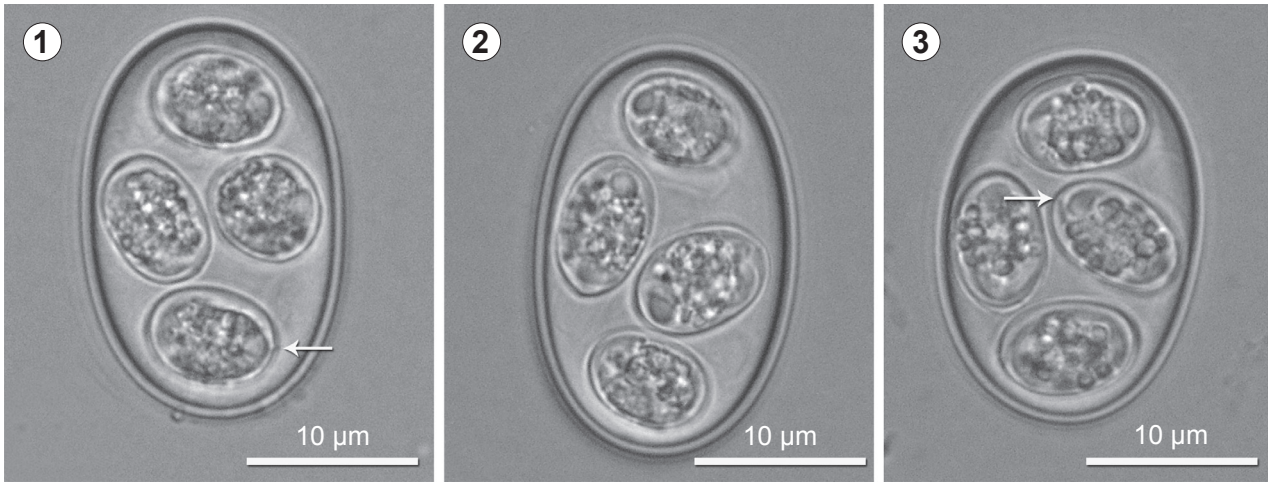


Figs. 1–3. Photomicrographs of freshly collected sporulated oocysts of *Choleoeimeria salaselensis* sp. n. from the gall bladder of the horned viper, *Cerastes gasperettii*; arrows indicates the sporocyst suture.

sues (stomach, intestine, gall bladder, liver, kidneys and muscles) were fixed in 10% neutral buffered formalin. Fixed tissues were processed for histology using standard methods; paraffin sections were stained with haematoxylin and eosin (H & E). All stages were observed and photographed using an Olympus BX51 microscope with an Olympus DP71 Camera. Measurements are given in µm as arithmetic mean followed by the range. Descriptions of oocysts and sporocysts follow guidelines of Duszynski and Wilber (1997) and Wilber et al. (1998).

RESULTS

All ten examined snakes were passing mostly sporulated oocysts of an undescribed species of coccidia, which we place into the genus *Choleoeimeria*, for reasons discussed later (see Remarks). The gall bladders of the infected snakes were cloudy and contained huge numbers of oocysts, but with no other signs of alteration in their health status.

Choleoeimeria salaselensis sp. n. Figs. 1–10

Synonym: *Eimeria phisalixae* of Vetterling and Widmer (1968) (see Remarks).

Description:

Exogenous stages (N based on 30 sporulated oocysts and sporocysts). Oocysts ellipsoidal, with a smooth, bilayered wall, 23 × 15 (22–25 × 14–17), with length/width ratio (L/W) 1.5 (1.4–1.6). Micropyle, polar granule and oocyst residuum all absent. Sporocysts ellipsoidal, 8 × 5 (7–9 × 5–6), with L/W 1.5 (1.4–1.6); sporocyst wall composed of 2 plates, joined by a longitudinal suture (Figs. 1, 3, 10). Stieda body, substieda body and parastieda body all absent. Sporocysts residuum present and composed of different sized granules. Sporozoites banana-shaped, usually lying head-to-tail, ~11 × 2, with 1 prominent refractile body.

Endogenous stages (N based on 10–20 tissue sections of meronts and gamonts). Endogenous development confined to distal part of epithelial cells lining bile duct and gall bladder. No endogenous stages encountered in any other organ. Infected host cells hypertrophied and displaced from epithelial layer into lumen and remain in contact with basal membrane only by a thin pedicle. Mature meronts irregular in shape, 11 × 9 (10–12 × 8–10), contain 10–16 merozoites (Figs. 4, 5). Nearly spheroidal microgamonts (Figs. 6–8) 12 (10–15) wide. Macrogamonts, mostly spheroidal (Figs. 5, 6–9) ~16 (15–18) wide, with prominent nucleus in centre and wall-forming bodies at their periphery (Fig. 9).

Type host: Desert horned or great cerastes viper, *Cerastes gasperettii* Leviton and Anderson (Serpentes: Viperidae).

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

Site of infection: Epithelial cells lining the bile duct and gall bladder.

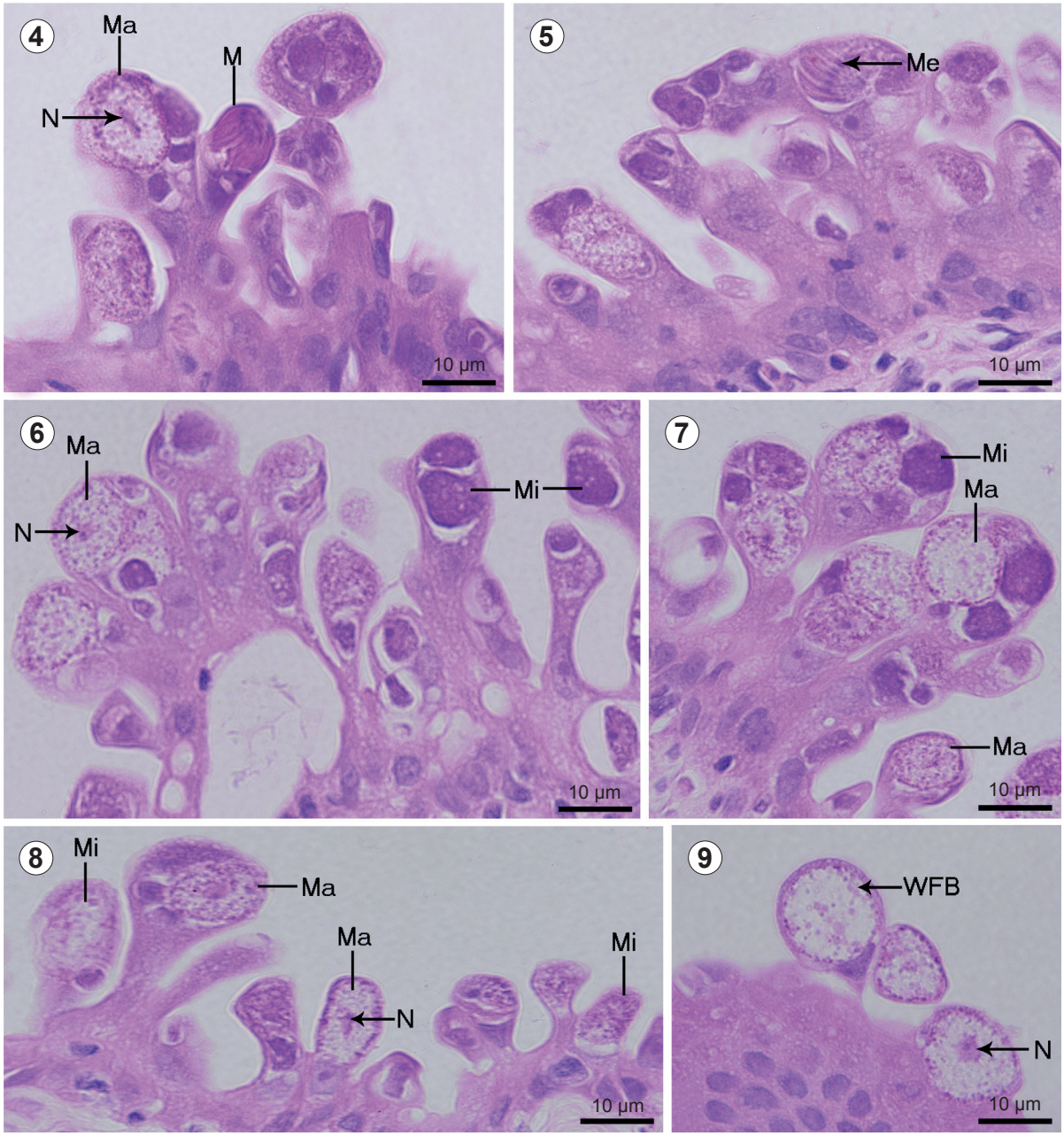
Prevalence: found in 10/10 (100%) hosts.

Sporulation: Sporulation endogenous and exogenous; both sporulated and unsporulated oocysts were found in the lumen of the gall bladder and intestinal contents prior to being shed to the faeces.

Type material: Photosyntype of the sporulated oocysts and one syntype slide of H & E-stained endogenous stages in the gall bladder epithelia have been deposited in the parasitological collection of the Hungarian Natural History Museum under the inventory number (HNHM 70397).

Etymology: The specific epithet *salaselensis* is derived from the host locality, Salasel.

Remarks. Two closely-related genera of coccidia, *Choleoeimeria* and *Eimeria*, are commonly found in reptiles, especially lizards and snakes; both have oocysts that contain four sporocysts, each with two sporozoites. Species of *Choleoeimeria* are defined by always having elongate-ellipsoidal to cylindroidal oocysts, undergoing partial to complete sporulation before being discharged in



Figs. 4–9. Endogenous stages of *Choleoeimeria salaselensis* sp. n. in the gall bladder epithelia of the horned viper, *Cerastes gasperettii*, photomicrographs. *Abbreviations*: M – mature meront; Ma – macrogamonts; Me – merozoites; Mi – microgamonts; N – centrally located nucleus; WFB – wall-forming bodies arranged at the periphery.

the faeces, and by having generally small, subspheroidal to ellipsoidal sporocysts that lack a Stieda body, substieda body and parastieda body, but have a longitudinal suture that separates into two plates during excystation (Paperna and Landsberg 1989, Jirků et al. 2002, 2009, Modrý and Jirků 2006, Sloboda and Modrý 2006, Abdel-Baki et al. 2008, 2009, 2013, Al-Quraishy 2011, McAllister 2012a,b).

The ellipsoidal sporulated oocysts we studied conformed in every way to *Choleoeimeria*, so we have placed our new species in that genus. However, there are five *Eimeria* species described from snakes of the family Viperidae (Table 1) that merit our consideration because of some similarities of their oocysts and sporocysts to those of *C. salaselensis*: *Eimeria amarali* Pinto, 1928, *E. bitis* Fantham, 1932, *E. cascabeli* Vetterling et Widmer, 1968, *E. cerastis* Chatton, 1912, and *E. gasperettii* Alyousif, Al-Anazi et Al-Shawa, 2003. However, all have much larger oocysts and somewhat larger sporocysts than the species we describe here as new. In addition, sporulated oocysts of *E. cascabeli* have a distinctly unilayered oocyst wall *vs* two layers we see in the oocysts wall of *C. salaselensis*.

Table 1. Comparative measurements (μm) of sporulated oocysts of *Choleoeimeria salaselensis* sp. n. and morphologically similar oocysts of currently described *Eimeria* species from snakes in the family Viperidae.

Species	Host	Country	Oocyst size range (mean)	Oocyst shape (length/width ratio)	Sporocyst size range (mean)	Sporocyst shape (length/width ratio)	Reference
Eimeria amarali	*Bothrops neuwiedi*	Brazil	27–34 × 17–19	ellipsoidal	9	spherical	Pinto 1928
Eimeria bitis	*Bitis arietans*	Namibia	27–37 × 17–25	ellipsoidal	8 (7–14) × 8 (7–14)	spherical	Fantham 1932
Eimeria cascabeli	*Crotalus oregonus helleri* *Crotalus viridis viridis*	USA	37 (34–40) × 17 (14–20)	ellipsoidal 2.2 (1.8–2.6)	10 (9–11)	spherical	Vetterling and Widmer 1968
Eimeria cerastis	*Cerastes gasperettii*	France	40 × 20	ellipsoidal	12 × 6	ellipsoidal	Chatton 1912
Eimeria gasperettii	*Cerastes gasperettii*	Saudi Arabia	39 (29–41) × 26 (18–28)	ellipsoidal 1.5 (1.4–18)	14 (11–15) × 10 (8–10)	ellipsoidal 1.4 (1.2–1.8)	Alyousif et al. 2003
Choleoeimeria salaselensis sp. n.	*Cerastes gasperettii*	Saudi Arabia	23 (22–25) × 15 (14–17)	ellipsoidal 1.5 (1.4 –1.6)	8 (7–9) × 5 (5–6)	ellipsoidal 1.5 (1.4–1.6)	Present study

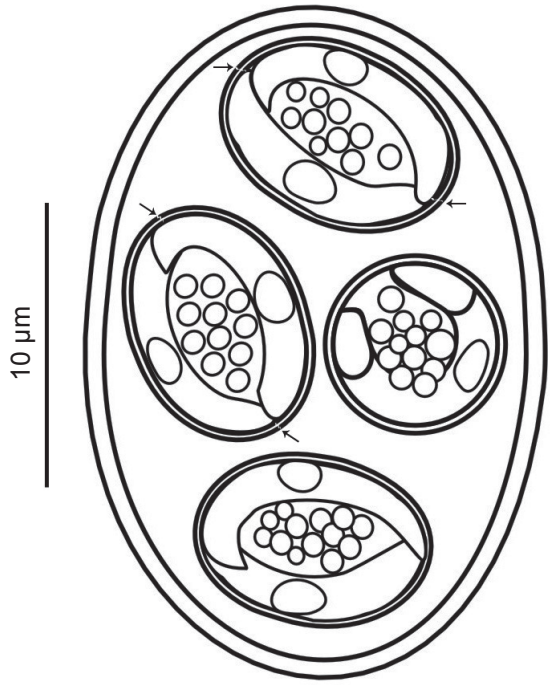


Fig. 10. Schematic drawing of an oocyst of *Choleoeimeria salaselensis* sp. n.; arrows indicated the sporocyst suture.

As pointed out by Duszynski and Upton (2009), Chatton (1912) first described oocysts he called *Eimeria cerastis* with oocysts that measured 40 × 20 μm and ellipsoidal sporocysts that were 12 × 6 μm. Later, Phisalix (1921) found oocysts in the bile and associated epithelial cells of the gall bladder that were 23 × 16.5 μm (21–25 μm × 15–18 μm) with spheroidal sporocysts ~10 μm wide, but she still called them *E. cerastis* for reasons known only to her. Vetterling and Widmer (1968), who reviewed her work, said she must have been dealing with a different species (with which we agree) and renamed the form described by Phisalix (1921) as *E. phisalixae*.

Unfortunately, none of the authors provided either a photomicrograph or a line drawing of the forms they saw and, as a result, Duszynski and Upton (2009) relegated *E. phisalixae* as a *species inquirenda*. We resurrect this issue because the measurements of *E. phisalixae* are similar to those of our new species, which was also described from *C. gasperettii* with only minor differences in sporocyst structure; our species has ellipsoidal sporocysts *vs* the round ones as described by Phisalix (1921). Actually, *E. phisalixae* was described from two oocysts, with not enough information to call this a valid species, as suggested by Duszynski and Upton (2009), and with which we agree. Based on the fact that we found oocysts in the gall bladder of *Cerastes* that are exceptionally close in morphology to the oocysts from the inadequate descriptions of *E. phisalixae* (see Phisalix 1921, Vetterling and Widmer 1968) we take this opportunity to synonymize *E. phisalixae* of Vetterling and Widmer (1968) with our form and to consider our species as new.

DISCUSSION

The generic classification of eimeriid coccidia reported from reptiles has been controversial because the vast majority of described and named species have been identified using only the morphology of their sporulated oocysts with little attention paid to the differences that might exist between the location(s) in the definitive host within which their endogenous development may occur (Jirků et al. 2009). The genus *Choleoeimeria* was proposed by Paperna and Landsberg (1989) for generally elongate-ellipsoidal oocysts, with sporocysts that lack a Stieda body and that developed in the epithelium of the gall bladder and bile duct of their hosts. Their proposed new genus did not gain immediate acceptance, but validity of the genus has been supported by molecular data (SSU rDNA –Jirků et al. 2002) and numerous subsequent studies (Modrý and Jirků 2006, Sloboda and Modrý 2006, Abdel-Baki et al. 2008, 2009, 2013, Jirků et al. 2009, Al-Quraishy 2011, McAllister 2012a,b).

Currently, it is unknown how many *Choleoeimeria* species exist for three reasons. First, the genus has only recently begun to be accepted as a valid taxon (with the conditions noted above) by those describing new forms from reptile hosts. Second, there are many *Eimeria*-like species named in the literature from reptiles that have el-

lipsoidal/cylindroidal-shaped oocysts and sporocysts with a longitudinal suture (unpublished data), but nothing is known about the location of their endogenous development; these have all been placed in the genus *Eimeria* until more can be learned about where their merogony and gamogony occur. Third, there are less than a handful of studies (most noted above) in which molecular sequencing has been done to help for generic placement.

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