Syst Parasitol (2013) 85:189–194
DOI 10.1007/s11230-013-9413-z

Redescription of *Eimeria zarudnyi* Alyousif & Al-Shawa, 2003 as *Choleoeimeria zarudnyi* n. comb. (Apicomplexa: Eimeriidae)

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Received: 31 December 2012 / Accepted: 8 March 2013

Abstract Coprological examination of the worm lizard *Diplometopon zarudnyi* Nikolskii revealed the presence of oöcysts of *Choleoeimeria zarudnyi* (Alyousif & Al-Shawa, 2003) n. comb. in five (17%) of the 30 lizards examined. Sporulated oöcysts were found in the faeces and the gallbladder contents. These are tetrasporocystic, ellipsoidal, 25–32 × 18–25 (mean 27 × 22) µm, with a smooth bi-layered wall. The dizoic sporocysts are ovoidal, 10–13 × 6–9 (mean 11 × 7) µm, with a granulated sporocyst residuum. Sporozoites are banana-shaped with an average size of 13 × 3 µm. Endogenous stages (meronts, gamonts and gametes) are confined to the gallbladder epithelium and the infected cells were hypertrophied. Based on the morphological features of the exogenous stages and the endogenous development of the present parasite, its generic affiliation is revised and *Eimeria zarudnyi* Alyousif & Al-Shawa, 2003 is transferred to the genus *Choleoeimeria*.

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Introduction

The genus *Eimeria* Schneider, 1875 comprises homoxenous coccidians possessing four dizoic sporocysts within the oöcyst. There are now more than 1,300 described species of *Eimeria* (see Duszynski et al., 2000) but the majority of them have been described based only on oöcyst morphology. Paperna & Landsberg (1989) separated the tetrasporocystic, dizoic oöcysts of reptiles into the genera *Eimeria*, *Choleoeimeria* Paperna & Landsberg, 1989 and *Acroeimeria* Paperna & Landsberg, 1989 (Protozoa: Eimeriidae), based on the site of infection and the mode of development of the endogenous stages. Species of the genus *Choleoeimeria* have elliptical oöcysts (length/width ratio 1.6–2.2), with endogenous development confined to the gallbladder epithelia and also lack a Stieda body.

Phylogenetic analysis based on nucleotide sequences of the small subunit ribosomal RNA gene has confirmed the status of the genus *Choleoeimeria* (see Jirků et al., 2002), indicating that it forms a sister clade to the family Eimeriidae. The generic separation of *Choleoeimeria* has thus become widely accepted and many new species belonging to this genus have been described or redescribed (e.g. Lainson & Paperna, 1999; Lainson, 2003; Modrý & Jirků, 2006; Sloboda & Modrý, 2006; Paperna, 2007; Abdel-Baki et al., 2008, 2009; Al-Quraishy, 2011; McAllister 2012a, b). These authors stressed the necessity of studying the endogenous stages of the eimeriid

coccidians in order to enable their allocation to their correct genus and species. According to Jirků et al. (2002), since it is impossible to properly classify the species that were originally described without notes on the site of infection and the presence or absence of sporocyst sutures and Stieda bodies, these species should be treated as *species inquirendae* or *incertae sedis* until more information is available. This has led Paperna (2007) to postulate that those eimerian species that have been reported in the literature as developing in the gallbladder epithelium should have their generic status amended. Following these suggestions, we here investigate the characteristics of the exogenous and endogenous stages for an eimeriid originally described as *Eimeria zarudnyi* Alyousif & Al-Shawa, 2003 from the gallbladder of the worm lizard *Diplometopon zarudnyi*. We provide a new combination and subsequently place the species in the genus *Choleoeimeria*.

Materials and methods

Thirty adult specimens of the worm lizard *Diplometopon zarudnyi* Nikolskii were collected during a parasitological survey in Saudi Arabia. Samples were captured by hand during June, 2012 from Al-Thumamah (24°41′N; 46°42′E) in Riyadh, in the central region of Saudi Arabia. The lizards were kept separately in plastic cages for several hours in order to collect faeces which were then macerated and examined for oöcysts. Fresh faeces were suspended in 2.5% (W/V) potassium dichromate solution. The oöcysts were concentrated using Sheather's sugar flotation technique. Heavily infected lizards were anaesthetized with diethyl ether, dissected, and bile was collected by puncturing the gallbladder with a finely pointed glass pipette. Selected tissues (stomach, intestine, liver, kidneys and muscles) were fixed in 10% buffered formalin. Fixed tissues were processed for histology using standard methods and paraffin sections were stained with haematoxylin and eosin. All stages were observed and photographed using an Olympus BX51 microscope with an Olympus DP71 camera according to the guidelines of Duszynski & Wilber (1997). Fifty sporulated oöcysts, and 10–20 endogenous stages were measured. All measurements are reported in micrometres; data are presented as the range followed by the mean ± standard deviation (SD) (where possible) in parentheses.

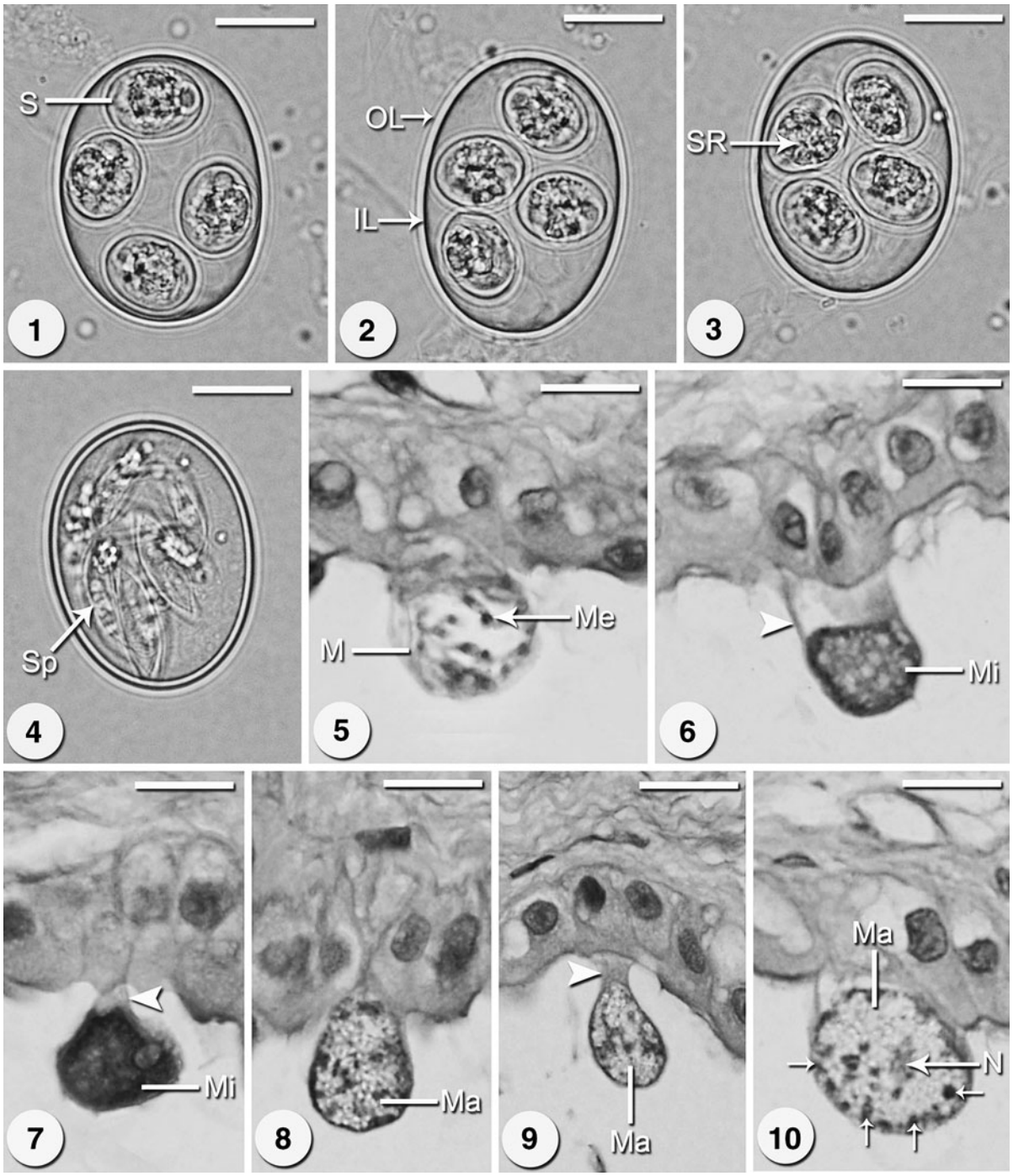
Results

Coprological examination revealed the presence of oöcysts of *Choleoeimeria* in five (17%) of the 30 lizards examined. This species is redescribed below.

Choleoeimeria zarudnyi (Alyousif & Al-Shawa, 2003) n. comb

Host: *Diplometopon zarudnyi* Nikolskii (Amphisbaenia: Trogonophidae) (type-host)
Locality: Al-Thumamah (24°41′N; 46°42′E), in Riyadh, in the central region of Saudi Arabia (type-locality).
Prevalence: 17% (5 out of 30 lizards).
Site of infection: Gallbladder.
Sporulation: Sporulation endogenous; both sporulated and unsporulated oöcysts were found in the gallbladder lumen and intestinal contents prior to being voided in the faeces.
Type-material: Photosyntype for the sporulated oöcysts and one slide with syntypes of haematoxylin-eosin stained endogenous stages in the gallbladder epithelia have been deposited in the parasitological collection of the Hungarian Natural History Museum under the inventory number HNHM-70392.

Description (Figs. 1–11)
Exogenous stages
Oöcyst without micropyle, elongate ovoid to ellipsoidal, rounded at ends, measuring 25–32 × 18–25 (27 × 22), with length/width ratio 1.0–1.3 (1.2) (Figs. 1–4, 11). Oöcyst surface smooth, wall bilayered; oöcyst residuum and polar granule absent (Figs. 1–4). Four oval sporocysts present, with thick single-layered wall and without Stieda body (Figs. 1–4). Dimensions of sporocysts 10–13 × 6–9 (11 × 7), with length/width ratio 1.3–1.6 (1.5). Sporocyst residuum composed of a large number of granules differing in size (Fig. 3). Sporozoites within sporocysts usually laying head to tail, with two refractile bodies (Fig. 11). Sporozoites banana-shaped, measuring on average 13 × 3 (from ruptured sporocysts, see Fig. 4).



Figs. 1–10 *Choleoeimeria zarudnyi* (Alyousif & Al-Shawa, 2003) n. comb. from the gallbladder of the worm lizard *Diplometopon zarudnyi*. 1–4. Photomicrographs of freshly collected mature oöcysts with bi-layered wall (OL, outer layer; IL, inner layer), containing four sporocysts (S) with sporocyst residuum (SR) and two sporozoites (Sp). 5–10. Endogenous stages. Infected epithelium becomes hypertrophied and displaced to the lumen of the gallbladder being connected with the basal membrane by a thin pedicle (arrowheads). 5. Mature meront (M) with mature merozoites (Me). 6, 7. Microgamonts (Mi). 8–10. Macrogamonts (Ma) with wall-forming bodies arranged at the periphery (arrows) and a centrally located nucleus (N). *Scale-bars*: 10 μm

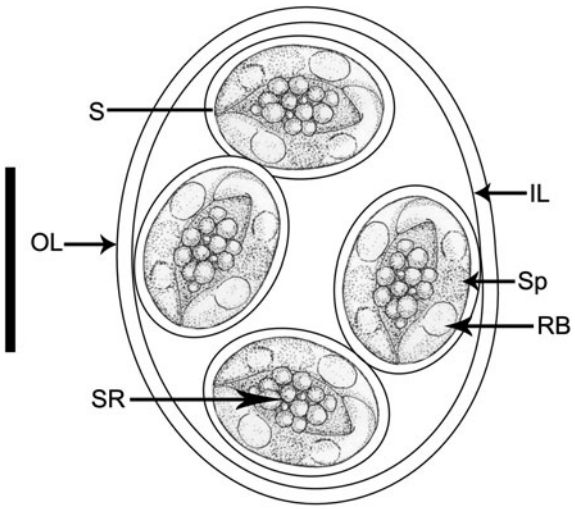


Fig. 11 Schematic drawing of a mature oöcyst of *Choleoeimeria zarudnyi* (Alyousif & Al-Shawa, 2003) n. comb. The oöcyst is with bi-layered wall (OL outer layer, IL inner layer) and contains four sporocysts (S), each with sporocyst residuum (SR) and two sporozoites (Sp) each with a refractile body (RB). *Scale-bar*: 10 μm

Endogenous development

Mature meronts spherical, measuring 12–15 (14 ± 0.6) in diameter, and estimated to produce 20–25 merozoites (Fig. 5). Microgamonts irregular in shape, measuring 9–12 × 7–10 (11 ± 0.4 × 8 ± 0.5) (Figs. 6, 7). Macrogamonts mostly subspherical (Figs. 8–10) with a prominent nucleus in the centre and wall-forming bodies at the periphery (Fig. 10), measuring 12–18 × 7–12 (14 ± 0.7 × 10 ± 0.8).

The intrinsic development occurred within epithelial cells lining the gallbladder and bile-duct. No endogenous stages were encountered in any other organ. The infected host cells were hypertrophied and protruded from the epithelial layer into the lumen (Figs. 5–10). Usually the infected cells maintained connection with the basal membrane only by means of a thin pedicle (Figs. 6, 7, 9).

Remarks

To our knowledge, three species of *Eimeria* and one species of *Choleoeimeria* have been described to date from among amphisbaenian lizards (Table 1). These are *Eimeria amphisbaeniarum* Huntington, Cisper, Smith, Powell, Parmerlee & Lathrop, 1996, *E. witchery* Huntington, Cisper, Smith, Powell, Parmerlee & Lathrop, 1996 associated with *Amphisbaena manni* Barbour (see Huntington et al., 1996), *E. zarudnyi* Alyousif & Al-Shawa, 2003 associated with *D. zarudnyi* (see Alyousif & Al-Shawa, 2003) and *Choleoeimeria amphisbaenae* Lainson, 2003 associated with *Amphisbaena alba* Linnaeus (see Lainson, 2003). *Eimeria amphisbaeniarum* and *E. witchery* have thinner oöcysts and a larger length/width ratio than those observed here, whereas the oöcysts of *C. amphisbaenae* are significantly longer and also have a larger length/width ratio. The morphology and measurements in the original description of *E. zarudnyi* are similar to those observed in the present material which is also described from the same host. Based on oöcyst morphology and identical host, we consider it conspecific with *E. zarudnyi*. Alyousif & Al-Shawa (2003) described *E. zarudnyi* on the basis of the morphology of oöcysts collected from the faeces. They suspected that the gallbladder was the site of infection and this was confirmed in the present study. Accordingly, the present biliary coccidium is classified as a member of the genus *Choleoeimeria* based on its oöcyst morphology and endogenous development.

Discussion

The descriptions of many species of *Eimeria* from reptiles are based solely on the morphology of their mature oöcysts and sporocysts following Levine (1973), with no consideration for any differences that might exist in the rest of the parasite life-cycles (Lainson, 2003). In general, oöcyst morphology can be quite diverse, both between and within host species, with the only constant feature being the presence of four sporocysts, each with two sporozoites (Asmundsson et al., 2001). It is impossible to be sure, therefore, of the generic status of many parasites without reference to the endogenous stages (Lainson & Paperna, 1999). Paperna & Landsberg (1989) proposed that the genus *Choleoeimeria* comprised some *Eimeria* and *Eimeria*-like coccidians infecting the biliary epithelium of reptiles.

Species of the genus *Choleoeimeria* have elliptical oöcysts (length/width ratio 1.6–2.2) with endogenous development confined to the gallbladder epithelia and lack a Stieda body. This separate generic status of the eimerians inhabiting gallbladders of reptilian hosts was not accepted until their distinctiveness from *Eimeria* spp. had been demonstrated through molecular

Table 1 Comparative measurements (in µm) of *Choleoeimeria zarudnyi* n. comb. and morphologically similar species from amphisbaenian lizards

Species	Host	Oöcyst size	Oöcyst shape (length/width ratio)	Sporocyst size	Sporocyst shape (length/width ratio)	Reference
		Range (mean)	Range (mean)	Range (mean)	Range (mean)	
Choleoeimeria amphisbaenae Lainson, 2003	*Amphisbaena alba*	30–37 × 20–26 (33 × 22)	Ellipsoidal to cylindrical 1.2–1.7 (1.5)	11–14 × 9–10 (13 × 9)	Ellipsoidal 1.2–1.5 (1.4)	Lainson (2003)
Eimeria witcheri Huntington & Cisper, 1996	*Amphisbaena manni*	22–29 × 15 –20 (26 × 18)	Ellipsoidal 1.3–1.6 (1.4)	9–12 × 7–9 (10 × 8)	Ellipsoidal 1.2–1.4 (1.3)	Huntington et al. (1996)
Eimeria amphisbaeniarum Huntington & Cisper, 1996	*Amphisbaena manni*	26–32 × 14–17 (29 × 15)	Cylindrical 1.6–2.1 (1.9)	8–12 × 5–8 (10 × 6)	Ellipsoidal 1.2–2.0 (1.6)	Huntington et al. (1996)
Eimeria zarudnyi Alyousif & Al-Shawa, 2003	*Diplometopon zarudnyi*	25–31 × 18–24 (27 × 22)	Cylindrical 1.1–1.4 (1.2)	10–12 × 6–8 (11 × 7)	Ellipsoidal 1.4–1.6 (1.5)	Alyousif & Al-Shawa (2003)
Choleoeimeria zarudnyi n. comb.	*Diplometopon zarudnyi*	25–32 × 18–25 (27 × 22)	Ovoid to cylindrical 1.0–1.3 (1.2)	10–13 × 6–9 (11 × 7)	Ellipsoidal 1.3–1.6 (1.5)	Present study

phylogenetic studies of Jirků et al. (2002). Subsequently, many authors (e.g. Lainson & Paperna, 1999; Lainson, 2003; Modrý & Jirků, 2006; Sloboda & Modrý, 2006; Paperna, 2007; Abdel-Baki et al., 2008, 2009; Al-Quraishy, 2011, McAllister 2012a, b) have stressed the necessity of studying the endogenous stages of these species in order to allocate them to their correct genus and species. Paperna (2007) also suggested that those eimerian species that have been reported in the literature as developing in the gallbladder epithelium should have their generic status amended. The current study, therefore, investigated the characteristics of the exogenous and endogenous stages of *E. zarudnyi* Alyousif & Al-Shawa, 2003 and revised its generic affiliation to the genus *Choleoeimeria*.

Acknowledgement The authors extend their appreciation to the Distinguished Scientist Fellowship Programme at King Saud University, Saudi Arabia for funding this study.

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