ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

A new species of *Choleoeimeria* (Apicomplexa: Eimeriidae) infecting the lizard *Chalcides sepsoides* (Sauria: Scincidae) in Egypt

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Manuscript Info

Manuscript History:

Received: 14 December 2013
Final Accepted: 21 January 2014
Published Online: February 2014

Key words:
Choleoeimeria, Apicomplexa, Eimeriidae, Sauria, Scincidae, lizard and *Chalcides sepsoides*.

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Abstract

Chalcides sepsoides collected from Nile Delta were infected with a new *Choleoeimeria* species. The prevalence of infection was 20 % (7/36). Oocysts were cylindrical, measuring 22.5 × 14 (20.6–24.7 × 11.8–15.9) µm. They were colorless with a smooth double–layered oocyst wall but lack micropyle, polar granules and oocyst residuum. Sporocysts were subspherical in shape, with a prominent meridional suture and measuring 7.6 × 6.5 (7.5–7.8 × 6.2–6.7) µm. Steida and substeida bodies were absent but sporocyst residuum, in the form of many coarse granules was observed. Each sporocyst contained two sporozoites. Sporozoites were banana shaped, each with a central nucleus and refractile body localized in one end. Sporozoite measured 10.6 × 2.0 (10.2–10.8 × 1.8–2.1) µm. When oocysts were preserved in 2.5% (w/v) potassium dichromate solution for about two weeks, excystation occurred and sporozoites were seen free within the oocyst. Endogenous development of the parasite was restricted to the biliary epithelium. Meronts at different stages of growth, microgamonts and macrogametes were described and measured. Hypertrophy of the infected host cells and their displacement above the epithelial layer was remarked.

Introduction

Scincid group is one of the most diversified saurians in Egypt, in spite of their large diversity, a few number of *Choleoeimeria* species have been described from these hosts (Abdel–Baki *et al*., 2013). *Choleoeimeria* is a coccidian genus, proposed by Paperna & landesberg (1989) including tetrasporocystic, dizoic and *Eimeria*–like coccidians infecting reptilian gall bladder epithelium. Establishment of this genus was confirmed by Jerků *et al*. (2002) via a phylogenic analysis based on nucleotide sequence of a small subunit ribosomal RNA gene.

Choleoeimerian oocysts are ellipsoidal to elongate and endogenously sporulate within the biliary lumen or in the intestinal lumen during their passage with faeces (Alyousif and Al–Rasheid, 2001; Abdel–Baki *et al*., 2008 & 2013). Also, sporocysts of *Choleoeimeria* lack Steida and substeida bodies and have a sporocyst wall composed of two valves attached together through a meridional suture. Moreover, hypertrohpy of infected host cells as well as their displacement into the lumen of gall bladder are also important characteristics of *Choleoeimeria* species (Paperna & Landesberg, 1989; Lainson & Paperna, 1999; Modry *et al*., 2001; Asmundsson *et al*., 2006; Paperna, 2007; Al–Nasr, 2011 and McAllister, 2012 a&b).

About 15 *Chloeoeimeria* species have been reported from members of family Scincidae (Table 1). Of these, nine have been described from the Middle East; three from USA; two from Australia and one from Japan. In the Middle East, six choleoeimerians were recorded from gall bladder of the members of genus *Scincus* which were *Choleoeimeria scinci* from *Scincus scincus* in Tunis (Phisalix, 1923); *C. chalcides* from *Chalcides ocellatus* in Egypt (Probert *et al*., 1988); *C. auratae* from *Mabouya aurata* in Saudi Arabia (Alyousif and Al–Rasheid 2001); *C. hemprichii* and *C. jazanensis* from *Scincus hemprichii* in Saudi Arabia (Alyousif and Al–Shawa, 2005 and Abdel–

Baki *et al*., 2013, respectively); *C. saqanqouri* from *Scincus scincus scincus* from Egypt (Abdel–Baki *et al*., 2008); *C. Baltrocki* from *Eumeces Schneidarii* in Egypt (Abdel–Baki *et al*., 2009); *C. riyadhae* from *Scincus scincus* in Saudi Arabia (Alyousif & Al–Shawa, 2010) and *C. mitranusensis* from *Scincus scincus* in Saudi Arabia (Al–Quraishy, 2011).

On the other side, some *Choleoeimeria* spp. including *C. scinci*, *C. hemprichii*, *C. auratae*, *C. egerniae*, *C. egregia*, *C. pellopleuris*, *C. scincellae*, *C. chlcides* and *C. fasciatus* infecting scincid hosts were firstly described belonging to genus *Eimeria*, later their systematic position was corrected by transferring them to genus *Choleoeimeria* by Modry and Jerků (2006).

The present work described exogenous and endogenous stages of *Choleoeimeria sepsoidi* n. sp. infecting the skink, *Chalcides sepsoides* from Nile Delta, Egypt using light microscopy.

Materials & methods

A total of 36 adult *Chalcides sepsoides* (Sauria, Scincidae) were collected from the desert of Beheira governorate, Nile Delta from March to August 2013. They were transferred to the laboratory, kept individually in separate plastic cages and immediately dissected. Fresh faeces were removed from the rectum. Oocysts of each infected lizard, were separately collected by floatation technique, microscopically examined and preserved in Petri dishes containing 2.5% (w/v) aqueous potassium dichromate solution.

To determine the infection site, smears of liver, gall bladder and mucosa of different parts of the alimentary canal were microscopically examined. The infected parts were gently removed and processed by the routine technique and sections of 5 µm thick were prepared and stained with haematoxylin and eosin (H&E). They were examined and various developmental stages of the parasite were measured and photographed using an Olympus BX41TF Japanese research light microscope.

Results

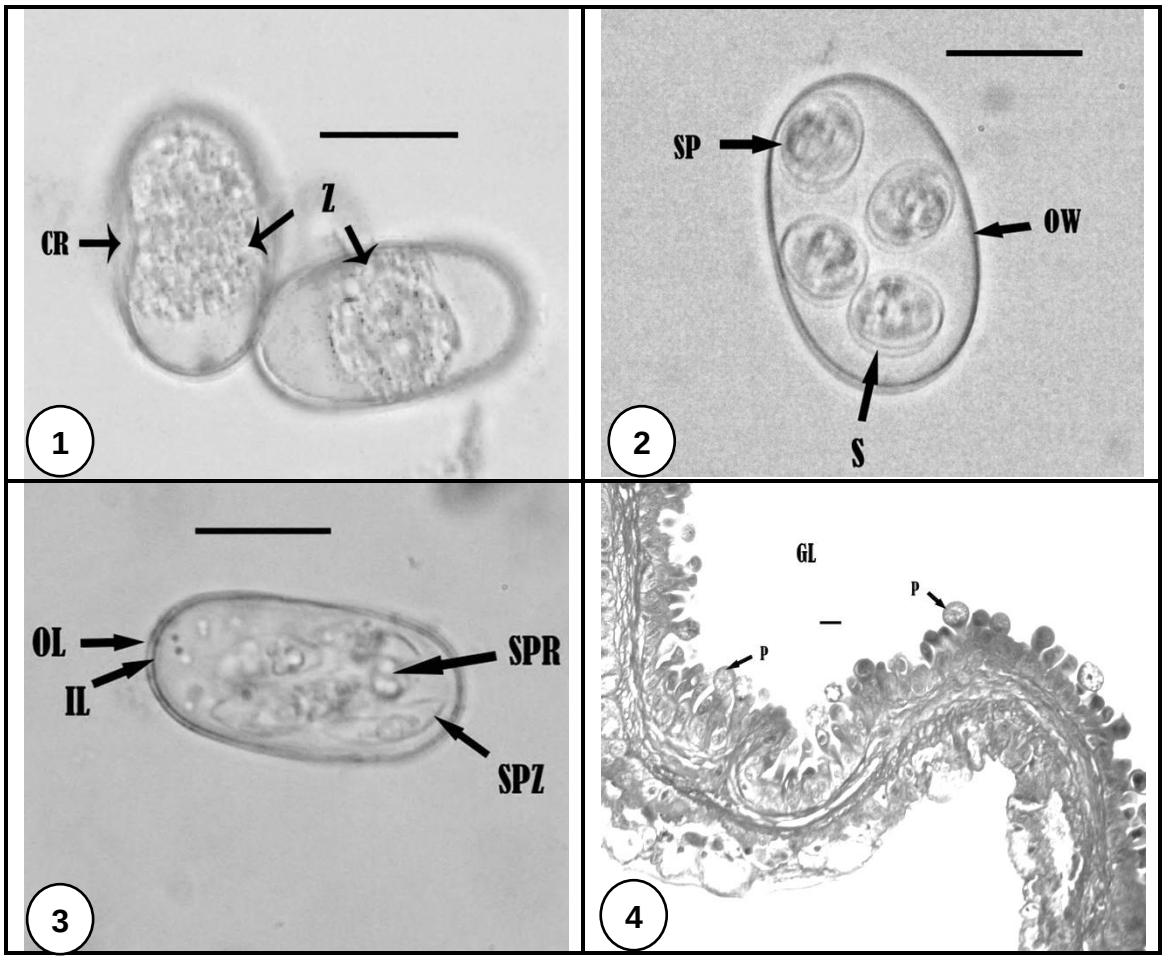
Exogenous stages

The majority of collected oocysts, either from gall bladder or from faeces were sporulated, however some of them were nonsporulated (Fig. 1). Sporulation was ascertained by the formation of two sporozoites within each sporocyst (Fig. 2).

Nonsporulated oocysts were colorless, each lined by a smooth, double–layered oocyst wall. The later consisted of an outer thin layer and an inner thick one which appeared darker in color. The oocyst contained a granulated, zygote filling most of the oocystic cavity, during sporulation zygote shrank and rounded in the middle of the cavity (Fig. 1). Nonsporulated as well as sporulated oocysts were of the same measurements 22.5 × 13.5 (20.6–24.7 × 11.8–15.9) µm. The length by width ratio (L/W) was 1.7 (1.6–2.0). Micropyle, polar granules oocyst residuum and other oocystic structures were absent (Figs. 1&2). Invagination of oocyst wall, at one side, was observed causing curvature appearance of oocysts.

Four sporocysts were recognized within the oocyst. Sporocysts (Fig. 2) were subspherical, each surrounded by a thin uni–layered wall which consisted of two valves adhered together by a meridional suture. Steida and substeida bodies were absent. Sporocyst residuum was found in the form of many coarse granules. Sporocysts measured 7.6 × 6.5 (7.5–7.8 × 6.2–6.7) µm; each contained two curved, banana–shaped sporozoites.

When the collected oocysts were left for about two weeks in 2.5% (w/v) potassium dichromate solution, sporozoites as well as many large granules of sporocyst residuum were seen free within the oocystic cavity (Fig. 3). Free sporozoites were banana–shaped (slightly curved), each with a large refractile body, measuring 10.6 × 2.0 (10.2–10.8 × 1.8–2.1) µm.



Figs. (1–3): Exogenous stages of *Choleoeimeria sepsoidi* n. sp.:
Fig. (1): Two nonsporulated oocysts, obliquily situated, the vertical one showed curvature of oocyst (CR) due to invagination of its wall at one side. Each oocyst contained zygote (Z) which exhibited the spherical shape at the beginning of sporulation. **Fig. (2):** Sporulated oocyst lined by an oocyst wall (OW) and contained four sporocysts (SP). Sporocyst wall was in the form of two valves attached together with a meridional suture (S). **Fig. (3):** Sporulated oocyst after two weeks in dichromate solution, note that excystation occurred within the oocyst and sporozoites (SPZ) became free in the oocyst cavity, the oocyst wall consisted of two layers, outer layer (OL) and inner darker one (IL).
Figs. (4&5): Site of endogenous growth of the parasite.
Fig. (4): Different stages of the parasite at the epithelial cells of gall bladder, the infected cells were displaced towards the gall bladder lumen (GL). The scale bar=10 µ.

Endogenous stages

Endogenous development of the parasite was found in epithelium of gall bladder and bile ducts (Figs. 4&5). Uninucleated meronts, each with a prominent nucleus were seen (Fig. 6) measuring 6.0 × 4.5 µm. Multinucleated meronts, with large nuclei distributed in the cytoplasm were also observed (Fig. 7), measuring 6.4 x 4.2 (6.3–7.0 × 4.0–4.6) µm. Mature meronts up to 20 having fully–formed merozoites could be seen (Fig. 8); each merozoite was banana–shaped, with a central nucleus, measuring 12.0 × 2.0 (11.8–12.1 × 1.8–2.1) µm. Young gamonts (Fig. 9) were characterized by having prominent nuclei; each surrounded with a pale cytoplasmic region and vary in size according to the degree of growth. Their measurements ranged from 5.3–8.2 µm in length and from 4.0–6.5 µm in width. Macrogametes (Fig. 11) were large in size, each with a central nucleus and wall–forming bodies arranged at the peripheral cytoplasmic surface. They measured 11.8 × 10.0 (11.5–12 × 9.7–10.2) µm. Microgamonts (Fig. 12) were recognized by their small nuclei arranged at the margins of their cytoplasm. They measured 13.5 ×12.9 (13.1–13.6 × 12.5–13.0) µm.

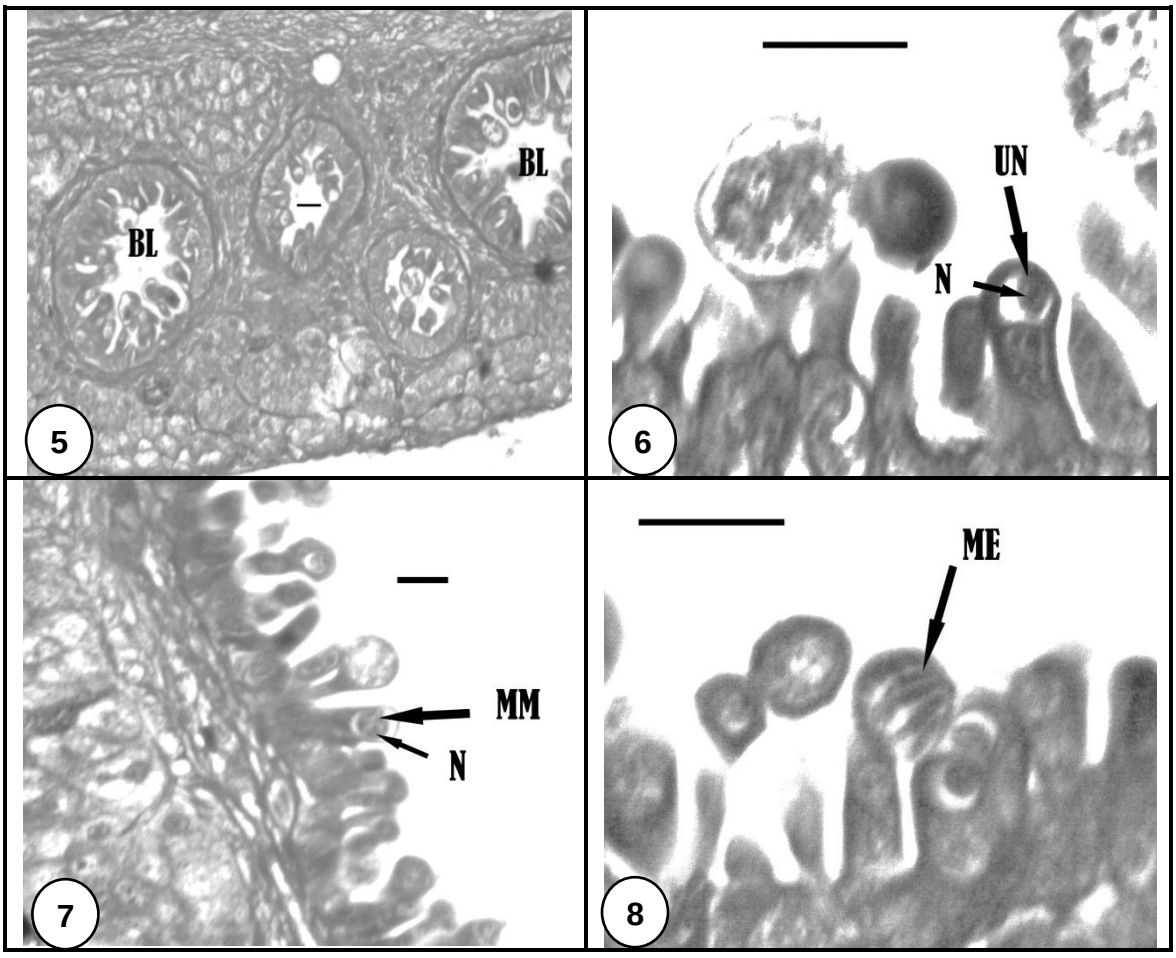


Fig. (5): Bile ducts showing biliary epithelial infection, the infected cells were displaced towards the bile-ducts lumen (BL).
Figs. (6–12): Endogenous stages of *Choleoeimeria sepsoidi* n. sp.
Fig. (6): Uninucleated meront (UN) with a prominent, central nucleus (N). **Fig. (7):** Multinucleated meront (MM) with four nuclei (N). **Fig. (8):** Mature meront, with fully–formed merozoites (ME). The scale bar=10 µ.

All endogenous stages developed in parasitophorous vacuoles within the infected host cells. The infected host cell was hypertrophied and protruded into the biliary lumen to be displaced over the non–infected neighboring ones (Figs. 5–12).

Discussion

Coccidians of family Eimeriidae parasitizing reptilian hosts, comprise essentially members of *Caryospora* Leger, 1904; *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Abdel–Baki *et al*., 2009). For a long time, it was accustomed to put tetrasporocystic, dizoic coccidians in genus *Eimeria*, depending only on the shape and measurements of both oocysts and sporocysts. However, some of the recognized *Eimeria* species from reptiles showed certain significant criteria including elongation of oocysts, bivalved sporocyst wall. Therefore, Genus *Eimeria* has been splitted into three genera: *Eimeria*, *Acroeimeria* and *Choleoeimeria*. The latter has ellipsoidal to cylindroidal oocysts, bivalved sporocyst wall, with a meridional suture, absence of Steida and substeida bodies in addition to specific infection site at the organic and cellular levels.

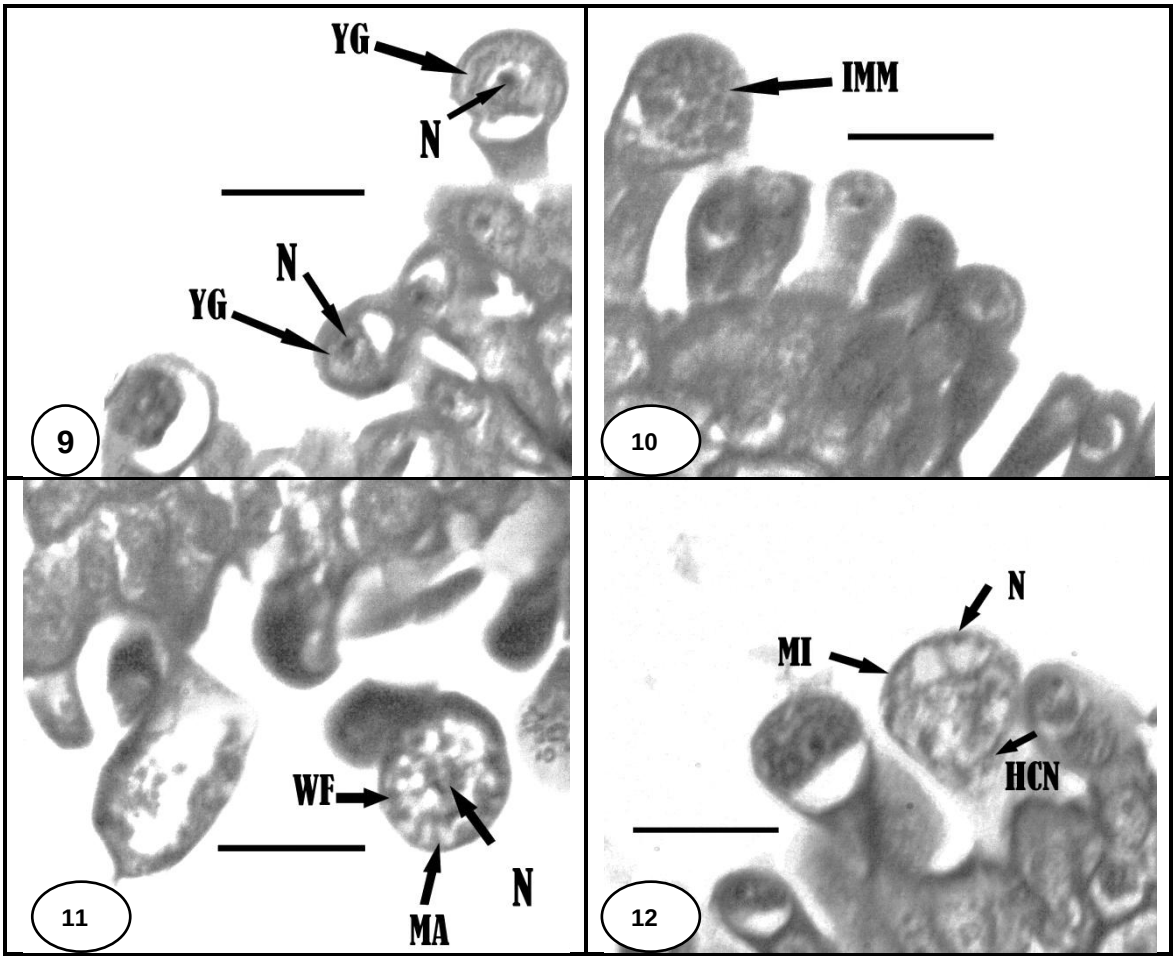


Fig. (9): Young gamonts (YG) at different stages of growth, the nucleus (N) was surrounded by a pale cytoplasmic region. **Fig. (10):** Immature gamont (IMM) with a highly granulated cytoplasm. **Fig. (11):** Macrogamete (MA) with a central nucleus (N) and wall–forming bodies (WF) at the margin of cytoplasm. **Fig. (12):** Microgamont (MI) with peripherally arranged nuclei (N), the host-cell nucleus (HCN) had a half- moon appearance. The hypertrophied host cells were displaced into the lumen and still attached to the basement membrane through a stalk of cytoplasm. The scale bar=10 µ.

Also, sporulation occurs endogenously in the biliary epithelium, causing hypertrophy of the infected host cells and their displacement into the gall bladder lumen (Paperna & Landesberg, 1989). The second genus is *Acroeimeria* with an epicytoplasmic development in the mucosa of digestive tract (Paperna&Landesberg, 1989). Whereas, genus *Eimeria* includes the members which develope in the cytoplasm of their host cells. When identifying a species of *Choleoeimeria*, it is reasonable to assume that the parasite might be able to infect different host species of the same genus, but not be transferred between different genera (Ball *et al*., 1994). Also, the same host genus might be infected with more than one parasite of the same species (as recorded from genera *Eumeces* and *Scincus*, Table 1).

The present parasite had all characters of members of genus *Choleoeimeria* previously described from the other reptiles (Paperna and Ladesberg, 1989; Alyousif and Al–Rasheid, 2001; Modry and Jerků, 2006; Abdel–Baki *et al*., 2008 & 2013; Alyousif and Al–shawa, 2010 and Al–Quraishy, 2011).

Curvature of the present oocysts, resulting from invagination of their walls was more distinct than irregular curved projections of folded inner layer of oocyst wall which were also visible inside the oocysts of *C. sadlieri* infecting the marble throated skink *Marmorosphax tricolor* from New Caledonia (Modry & Jerků, 2006) and *C. baltrocki* infecting the gall bladder of gold skink *Eumeces schneidarii* from Egypt (Abdel–Baki *et al*., 2009). Oocyst residuum and polar granules were absent in the present oocysts which distinguished them from that of *Choleoeimeria sadlieri* (Modry&Jerků, 2006).

Table (1): Comparison between *Choleoeimeria* species from members of Family Scincidae

Species	Host	Oocyst		Sporocyst		Locality	Reference
		shape	size	shape	size		
Choleoeimeria scinci	*Scincus scincus*	Cylindrical	25.0-36.0 long	------	14.0 x 10.0	Tunis	Modry & Jerků, 2006
C. egerniae	*Egernia whitii*	Cylindrical (?)	30.3 (28.0-32.0) x 16.1 (15.0-17.0)	Ellipsoidal (?)	10.2 (9.1-11.2) x 6.6 (6.0-7.1)	Australia	
C. pellopleuris	*Lygosoma pallopleurum*	Cylindrical (2.21)	31 (28-35) x 14 (12-15)	Ellipsoidal (1.26)	9.0 (8.0-10) x 7.0 (6.0-7.0)	Japan	
C. chalcides	***Chalcides ocellatus***	**Cylindrical (1.88)**	**35.0 (32.0-37.0)** x **18.6 (17.0-20.5)**	**Broadly ellipsoidal (1.35)**	**11.9 (8.5-13) x 8.9 (7.5-11)**	**Egypt**	
C. fasciatus	*Eumeces fasciatus*	Cylindrical (1.9-2.3)	34.9 (32.0-36.2) x 16.2 (15.2-17.4)	Ellipsoidal (1.4)	12 (10.4-13.2) x 8.7 (8.0-9.4)	USA	
C. egregia	*Eumeces egregious onocrepis*	Oval (1.59)	27.6 (25.0-32.0) x 17.4 (16.0-20.0)	Oval (1.24)	10.3 (8.5-12) x 8.3(7.0-9.0)		
C. scincellae	*Scincella lateralis*	Cylindrical (1.89)	29.8 (28.0-33.0) x 15.9 (14.0-17.0)	Oval (1.36)	10.9 (9.5-12.0) x 8.0 (7.0-9.0)		
C. auratae	*Mabouya aurata*	Cylindrical (1.5)	27.7 (22.0-31.5) x 18.5 (13.5-21.5)	Ellipsoidal (?)	11.8 (10.5-12.8) x 8.5 (7.5-9.0)	Saudi Arabia	
C. sadlieri	*Marmorosphax tricolor*	Cylindrical (2.12)	35.2 (32.0-39.0) x 16.7 (13.0-18.0)	Irregular (1.16)	12.0 (11.0-13.0) x 10.4 (9.0-11.0)	New Caledonia	
C. saqanqouri	*Scincus scincus scincus*	Ellipsoidal (1.5)	35.0 (33.0.5-37.0) x 23.5 (22.0-25.0)	Ellipsoidal (1.3)	11.5 (10.5-12) x 9.0 (7.5-10.0)	Egypt	Abdel–Baki *et al*., 2008
C. baltrocki	*Eumeces schneiderii*	Cylindrical (1.96)	38.7(36.0-42.0) x 19.9 (17.0-25.0)	Broadly ellipsoidal (1.2)	10.8 (9.5-13) x 9.3 (8-10.5)	Egypt	Abdel-Baki *et al*., 2009
C. riyadhae	*Scincus scincus*	Broadly ellipsoidal (1.21)	36.8 (33.4-39.1) x 30.5 (28.7-32.5)	Elongate ellipsoidal (1.63)	14.8 (13.7-15.5) x 9.1 (8.1-10.5)	Saudi Arabia	Alyousif & Al-Shawa, 2010
C. mitranusensis	*Scincus mitranus*	Ellipsoidal (1.4)	29.0 (28.0-31.0) x 20.0 (19.0-21.0)	Ellipsoidal (1.3)	11.0 (9.0-12.0) x 8.0 (7.0-9.0)	Saudi Arabia	Al-Quraishy, 2011
C. jazanensis	*Scincus hemprichii*	Cylindrical (1.7)	26.0 (25.0-27.0) x 15.0 (14.0-16.0)	Subspherical (1.3)	10.0 (9.0-11.0) x 7.0 (6.0-8.0)	Saudi Arabia	Abdel-Baki *et al*., 2013
***C. sepsoidi* n. sp.**	***Calcides sepsoides***	**Cylindrical (1.70)**	**22.5 (20.6-24.7) x 14.0 (11.7-15.9)**	**Ellipsoidal (1.20)**	**7.6 (7.5-7.8) x 6.5 (6.2-6.7)**	**Egypt**	**The present study**

The infected host cells in the present investigation were hypertrophied and displaced from the epithelial layer to above the surface and the attachment area between parasitized cells and also the underlined basement membrane became reduced to a narrow peduncle. These results were also reported in the previously described choleoeimerians (Abdel–Baki *et al*., 2009).

However, both oocysts and sporocysts of the present *Choleoeimeria* species were markedly smaller in size and did not overlap with oocysts and sporocysts of the previously described choleoeimerians from the other scincid hosts except *Choleoeomeria jazanensis* from *Scincus hemprichii* in Saudi Arabia (Abdel–Baki *et al*., 2013). Since no data about cross transmission were published and the parasite could not pass the generic boundaries (Ball *et al*., 1994), the present *Choleoeimeria* could not be considered *Choleoeimeria jazanensis*.

Furthermore, since the present *Choleoeimeria* species infect *Chalcides sepsoides*, it was also necessary to compare the present data with those of *Choleoeimeria chalcides* infecting *Chalcides ocellatus* (Table 1). The comparison indicated that oocysts, sporocysts and sporozoites of *Choleoeimeria* of the present parasite were much smaller than those of *C. chalcides.*

Considering the above mentioned discussion according to the comparative data in Table (1) in addition to the differences in the host species among the previously mentioned choleoeimerians and the present parasite, it is obvious that the latter is a distinct and hitherto undescribed species. So, the present authors considered the present *Choleoeimeria* a new species. It is suggested to be named *Choleoeimeia sepsoidi*.

Taxonomic summary

***Choleoeimeia sepsoidi* n. sp.**

Type host: *Chalcides sepsoides*.

Type locality: Beheira, Nile Delta, Egypt.

Prevalence: 20% (7/36).

Site of infection: Gall bladder.

Sporulation: Majority of oocysts sporulated endogenously, however some of nonsporulated oocysts were observed.

Type materials: Phototypes and histological sections with endogenous developmental stages were deposited at Zoology Department, Faculty of Science, Menoufia University.

Pathology: All infected lizards had a healthy appearance; however the infected biliary cells were hypertrophied and displaced into the lumen.

Etymology: The species name of the parasite was derived from the species name of the host.

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