

## ***Choleoeimeria bunopusi* sp. n. (Apicomplexa: Eimeriidae) Infecting the Gall Bladder of the Tuberculated Gecko *Bunopus tuberculatus* (Reptilia: Gekkonidae) from Saudi Arabia**

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**Abstract.** *Choleoeimeria bunopusi* sp. n. is described from the gall bladder of the tuberculated gecko *Bunopus tuberculatus* in Saudi Arabia. The prevalence of infection was 13.3% (2/15). Oocysts were ellipsoidal and measured 31 (30–33) × 21 (20–22) μm. Sporocysts were dizoic, elliptical in shape and measured 12 (11–13) × 7 (6–8) μm. The endogenous development was confined to the gall bladder epithelium. The hypertrophic parasitized biliary epithelium either remained in one layer or became stratified. Meronts, gamonts, and young oocysts were detected.

**Key words:** Coccidia, oocyst, *Choleoeimeria*, endogenous stages, Gekkonidae, Sauria.

### INTRODUCTION

Coccidians of the family Eimeriidae have been recorded in all classes of vertebrates, including reptiles (Lainson *et al.* 2008). In general, the taxonomy of the reptilian eimeriids coccidia is controversial (Jirků *et al.* 2002). Traditionally, their classification into genera and families is based on the morphology of the exogenous stages (oocysts) and on the endogenous stages of the life cycle (Jirků *et al.* 2002, Abdel-Baki *et al.* 2008). Many eimeriids coccidian species parasitizing

reptiles have been identified according to the morphology of their oocysts only, however, regardless of their endogenous development (Abdel-Baki *et al.* 2009). Modrý and Jirků (2006) and Abdel-Baki *et al.* (2009) have stressed the necessity of studying the endogenous stages of eimeriid coccidia as a fundamental tool in allocating them to a particular genus.

Paperna and Landsberg (1989), meanwhile, gave a new generic name, *Choleoeimeria*, to those Eimeriidae in reptiles which underwent a peculiar development in the biliary epithelium of the host. Species of the genus *Choleoeimeria* are biliary *Eimeria*-like coccidia of reptiles. Morphologically they are similar to the *Eimeria* to which they are closely related. Oocysts of all the described species of *Choleoeimeria* do share similar basic characteristics in their endogenous development

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in the biliary epithelium, sporulation in the gall bladder, their elongated oocysts, and the presence of four dizoic sporocysts which lack Stieda and sub-Stieda bodies and possess a bi-valved structure (Lainson 2003). Accordingly, herein we describe the present biliary coccidium as a new species of the genus *Choleoeimeria* from the gall bladder of *Bunopus tuberculatus*, based on the morphology of its oocysts and its endogenous development.

## MATERIALS AND METHODS

Fifteen adult tuberculated gecko *Bunopus tuberculatus* Blanford, 1874 were collected during a parasitological survey in Al-Thumamah (24°41'N, 46°42'E), in Riyadh, in the central region of Saudi Arabia during July and August, 2012. These animals were caged separately and infection was detected by demonstration of oocysts in the faeces. Positive geckos were then killed by chloroform and the infection in the gall bladder was verified by microscopic examination of bile collected by puncturing the gall bladder with a finely pointed glass pipette. To determine the site of infection, the entire gall bladder of each animal was then fixed in 10% neutral buffered formalin, together with portions of liver, spleen, kidney, lung, and intestine. Fixed tissues were processed for histological examination; they were sectioned and stained with hematoxylin and eosin (H & E). All the developmental stages were observed and photographed using an Olympus BX51 microscope with an Olympus DP71 camera. Measurements were made using an eyepiece micrometre with an oil-immersion lens; they are given here in micrometres ( $\mu\text{m}$ ; mean  $\pm$  standard deviation), followed by the range in parentheses and the length/width ratio.

## RESULTS

The prevalence of infection was 13.3% (2/15). The infected gall bladder was a deep blue colour, and the bile was turbid due to the presence of large numbers of free-floating oocysts at different stages of maturation with associated cell debris.

### Description

#### *Choleoeimeria bunopusi* sp. n. (Figs 1–9)

Mature oocysts were transparent and appeared ellipsoidal in shape (Figs 1–5, 9). They measured  $31 \pm 0.7$  (30–33)  $\times$   $21 \pm 0.6$  (20–22)  $\mu\text{m}$  with length/width ratio 1.5 (1.4–1.6)  $\mu\text{m}$ . The oocyst wall was smooth, about 1.0  $\mu\text{m}$  thick, colourless and with no micropyle or striations (Figs 2–4). This wall was composed of two layers: an outer, very fine, membrane and a thicker inner

one (Figs 3, 4). There was no oocyst residuum or polar body. Each oocyst contained four dizoic sporocysts (Figs 1–4). They were elliptical in shape with no Stieda body. These sporocysts measured  $12 \pm 0.6$  (11–13)  $\times$   $7 \pm 0.5$  (6–8)  $\mu\text{m}$  length/width ratio 1.4 (1.3–1.6)  $\mu\text{m}$ . The sporozoites were banana-shaped (Figs 4, 5) and measured  $14 \times 4 \mu\text{m}$ . Histological examination revealed all endogenous stages, including meronts, macrogamonts, and microgamonts in the gall bladder epithelium of infected lizards (Figs 6–8). No endogenous stages were observed in other organs. Infected epithelial cells were displaced from the mucosa toward the lumen, usually maintaining contact with the basal membrane only by a thin pedicle (Figs 7, 8). The hypertrophic parasitized biliary epithelium either remained in one layer (Figs 6, 7) or became stratified (Fig. 8). Mature meronts were nearly rounded to elliptical in shape, measured  $16$  (15–17)  $\times$   $12$  (11–13)  $\mu\text{m}$  and were estimated to produce 9–15 merozoites (Figs 6, 8). Mature microgamonts measured approximately  $18$  (17–19)  $\times$   $15$  (14–16)  $\mu\text{m}$  and were estimated to produce over 50 microgametes (Fig. 7). Macrogamonts were mostly spherical and measured  $16$  (15–18)  $\mu\text{m}$  in diameter (Fig. 8).

### Taxonomic summary

**Type host:** *Bunopus tuberculatus* Blanford, 1874.

**Type locality:** Al-Thumamah (24°41'N, 46°42'E), in Riyadh, in the central region of Saudi Arabia.

**Prevalence:** Two of 15 (13.3%).

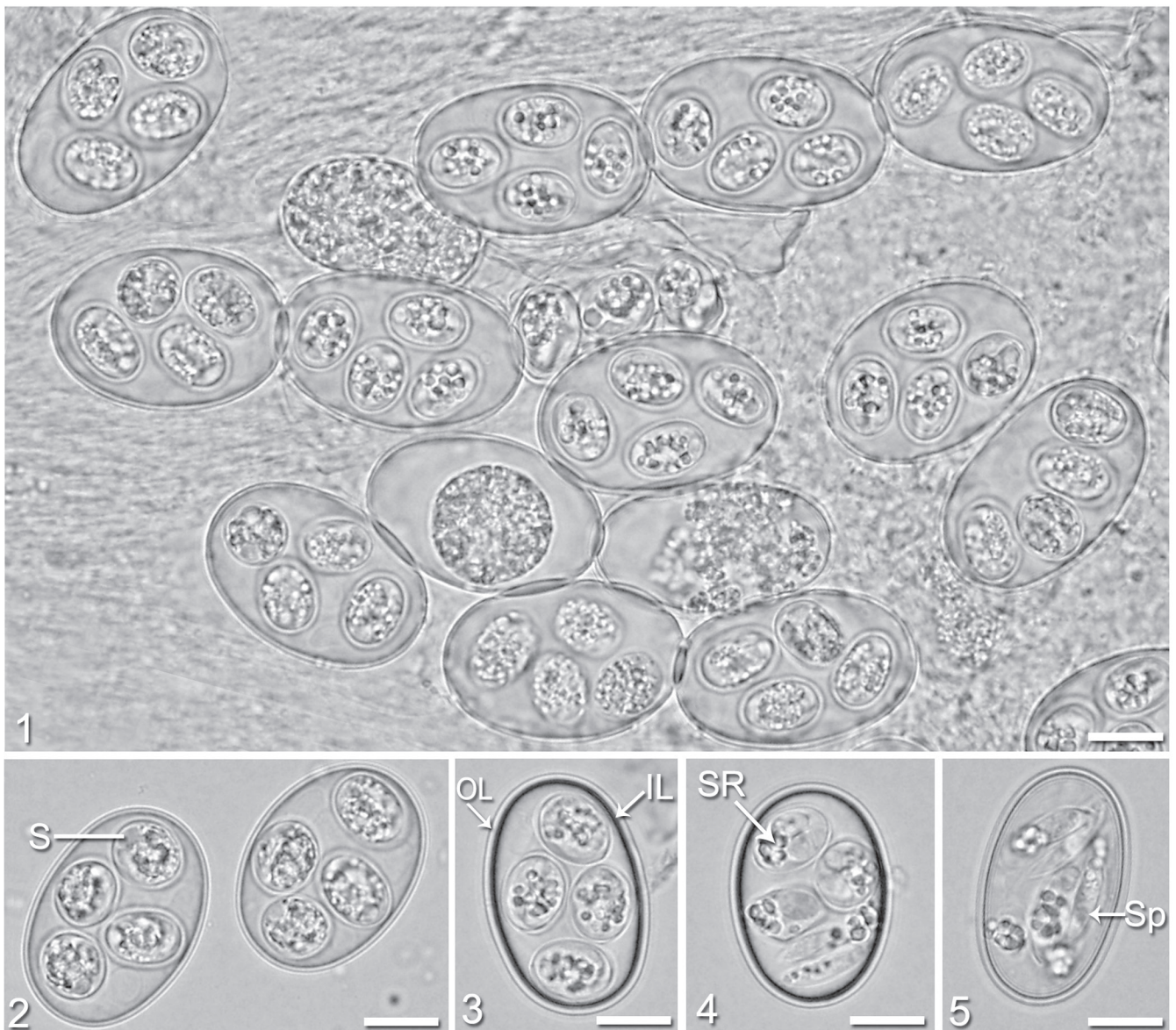
**Site of infection:** Gall bladder.

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

**Type material:** Photosyntypes of the sporulated oocysts and syntypes of oocysts of *Choleoeimeria bunopusi* in gall bladder content of *Bunopus tuberculatus* – sample in 80% ethanol are deposited at Protistological Collection of the Inst. of Parasitology ASCR, Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic with associated collection number: IP ProtColl P22.

As well as Photosyntypes and one slide of H & E stained endogenous stages in the gall bladder epithelia are deposited in Hungarian Natural History Museum with associated collection number: Par. Lab. HNHM-70393.

**Etymology:** The specific epithet is derived from the host name *Bunopus tuberculatus*.

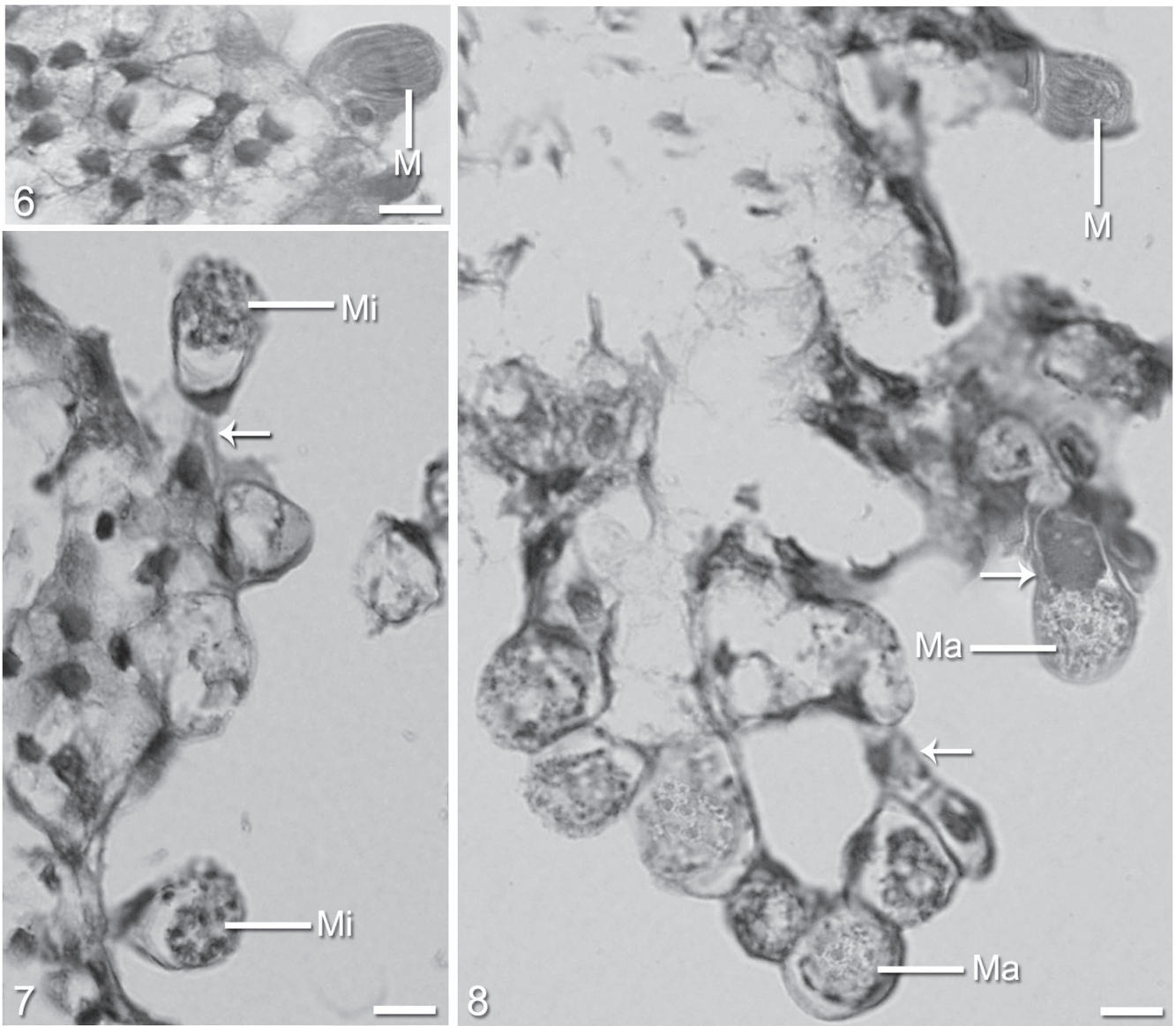


**Figs 1–5.** Photomicrographs of freshly collected oocysts of *Choleoeimeria bunopusi* sp. n. in different stages of development obtained from the gall bladder of *Bunopus tuberculatus*. Mature oocysts surrounded with outer layer (OL) and inner layer (IL) membrane and containing four sporocysts (S). Each sporocyst have two sporozoites (Sp) with sporocyst residuum (SR). Scale bars: 10  $\mu$ m.

### Remarks

According to Duszynski and Wilber (1997), a new coccidian species should be compared in detail with other coccidian species that are feature-similar and belong to the same host family. So far, six *Choleoeimeria* species have been described from the family Gekkonidae hosts (Table 1). These species include *Choleoeimeria faviviridis* Setna and Bana 1935 (Paperna and Landsberg 1989); *Choleoeimeria turcicus* Upton *et al.*

1988 (Paperna and Landsberg 1989), *Choleoeimeria pachydactyli* Paperna and Landsberg 1989; *Choleoeimeria rochalimai* Carini and Pinto 1926 (Lainson and Paperna 1999); *Choleoeimeria xiangmaii* Paperna 2007 and *Choleoeimeria heteronotis* Paperna 2007. Comparing these species, *C. faviviridis* have thinner oocysts (11–14 vs. 17–20) with a larger length/width ratio (1.8–2.4 vs. 1.4–1.6) and smaller sporocysts (7–9 vs. 11–13), while *C. turcicus* have relatively longer oocysts (35–41 vs. 30–33) and a relatively larger oocyst length/



**Figs 6–8.** Endogenous stages of *Choleoeimeria bunopusi* sp. n. at different stages of development in the epithelium of the gall bladder of *Bunopus tuberculatus*. **6** – mature meront (M); **7** – microgamont (Mi); **8** – macrogamont (Ma). Arrows refer to the infected epithelial cells that remain in contact with the basal membrane by a thin pedicle. Scale bars: 10  $\mu$ m.

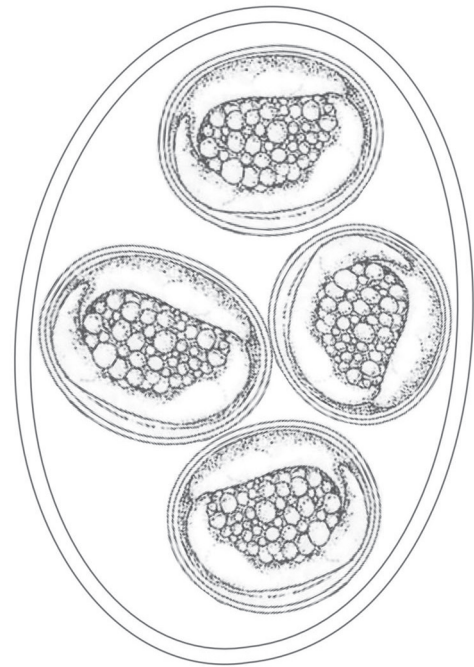
width ratio (1.9–2.3 vs. 1.4–1.6). Similarly, *C. pachydactyli* have thinner oocysts (11–17 vs. 20–22) with a relatively larger length/width ratio (2.05 vs. 1.5). The oocysts of *C. pachydactyli* have a small knob occurring at one pole. Although, *C. rochalimai* have a very similar oocyst size to that observed here, they differ in that their oocysts frequently possess a conspicuous polar body and have shorter pear-shaped sporocysts (7.5–10.0 vs. 11–13). Meanwhile, *C. xiangmaii* and *C. heteronotis* can be differentiated by their thinner oo-

cysts (12.5–16.2, 16.2–17.5 respectively vs. 20–22), larger oocyst length/width ratios (1.77–2.60, 1.85–2.10 respectively vs. 1.4–1.6) and shorter sporocysts (7.5–10.0, 8.7–10.0 respectively vs. 11–14). During the present study, endogenous stages were contained either in a single layer bile epithelium or a stratified one. In some geckoes *Choleoeimeria* were found to exhibit a single layer of infection, as in the case of *C. xiangmaii* and *C. heteronotis* (Paperna 2007). In other geckoes, however, infection induces stratified rather than single layered

**Table 1.** Comparative descriptive measurements ( $\mu\text{m}$ ) of *Choleoeimeria bunopusi* sp. n. with morphologically similar species from hosts of the family Gekkonidae.

Species	Host	Oocyst shape [L/W]	Oocyst size	Sporocyst shape [L/W]	Sporocyst size
<i>Choleoeimeria faviviridis</i> (Setna and Bana 1935) Paperna and Landsberg 1989	<i>Hemidactylus faviviridis</i>	(?) 1.8–2.4	25–34 $\times$ 11–14	(?/?)	7–9 $\times$ 5–7
<i>Choleoeimeria turcicus</i> (Upton <i>et al.</i> 1988) Paperna and Landsberg 1989	<i>Hemidactylus turcicus</i>	Cylindrical 2.1 (1.9–2.3)	38.2 (35–41) $\times$ 17.9 (17–20)	Spherical to ellipsoidal (?)	11 (10–12) $\times$ 8.8 (8.4–9.4)
<i>Choleoeimeria pachydactyli</i> Paperna and Landsberg 1989	<i>Pachydactylus capensis</i>	Cylindrical 2.05	28.3 (25–31) $\times$ 13.9 (11–17)	(?/?)	11.4 (10.14–12.6) $\times$ 6.9 (6.5–7.2)
<i>Choleoeimeria rochalimai</i> (Carini and Pinto 1926) Lainson and Paperna 1999	<i>Hemidactylus mabouia</i>	Cylindrical 1.6 (1.3–1.7)	29.1 (26.2–31.2) $\times$ 17.8 (16.2–18.7)	Pear-shaped (?)	9.0 (8.7–10.0) $\times$ 7.5 (6.2–7.5)
<i>Choleoeimeria xiangnani</i> Paperna 2007	<i>Hemidactylus fernatus</i>	Oblong 1.97 (1.77–2.60)	29.7 (28.7–32.5) $\times$ 15.1 (12.5–16.2)	Ellipsoidal to subspherical (?)	9.3 (7.5–10.0) $\times$ 5.7 (5.0–7.5)
<i>Choleoeimeria heteronotis</i> Paperna 2007	<i>Heteronotta bimoei</i>	Oblong 1.94 (1.85–2.10)	32.8 (32.5–33.7) $\times$ 16.9 (16.2–17.5)	Ellipsoidal 1.3 (1.1–1.6)	9.9 (8.7–10.0) $\times$ 7.6 (6.2–7.7)
<i>Choleoeimeria bunopusi</i> sp. n. (the present study)	<i>Bunopus tuberculatus</i>	Ellipsoidal 1.5 (1.4–1.6)	31 (30–33) $\times$ 21 (20–22)	Ellipsoidal 1.3 (1.2–1.5)	12 (11–13) $\times$ 7 (6–8)

? No data, [L/W] length/width ratio

**Fig. 9.** Composite line drawing of sporulated oocyst. Scale bar: 10  $\mu\text{m}$ .

bile epithelium, as in the case of *C. faviviridis* (Paperna and Landsberg 1989) and *C. rochalimai* (Lainson and Paperna 1999).

## DISCUSSION

Bovee and Telford (1965) observed two distinct groups of *Eimeria* among lizards based on differences in the shape of the oocyst as expressed by its length/width ratio. The first group has ellipsoidal or cylindrical oocysts with a length/width ratio ( $> 1.4$ ). This group also exhibit endogenous sporulation and undergo endogenous development in the gall bladder or biliary ducts. The second group, on the other hand, has spherical or rounded oocysts with a length/width ratio ( $< 1.4$ ). This group exhibits exogenous sporulation with the endogenous stages inhabiting the intestinal epithelium.

In 1989, Paperna and Landsberg proposed genus *Choleoeimeria* as a new genus to comprise some tetrasporocystic *Eimeria*-like coccidia infecting the gall bladders of reptiles. Use of this new genus has not gained wide acceptance because we know very little about the endogenous location and development of the

vast majority of named *Eimeria* species (Assmundsson *et al.* 2006). Recently, however, molecular studies have increased the support for the new genus. For example, Morrison (2009) has shown that *Eimeria* are paraphyletic and that the single species currently grouped within genus *Eimeria* should in fact be placed in several different genera. Furthermore, phylogenetic analysis, based on the nucleotide sequence of small sub-unit ribosomal RNA genes, has confirmed the separate status of the genus *Choleoeimeria* (Jirku *et al.* 2002).

In the present work, we have demonstrated that the gall bladder was the only site for the endogenous development of *C. bunopusi* sp. n. and that no endogenous stages were detected in intestine. Therefore, we have followed Paperna and Landsberg (1989) and Modry and Jirku (2006) in classifying the biliary coccidium described in this study as a member of the genus *Choleoeimeria*.

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