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COCCIDIA (APICOMPLEXA: EIMERIIDAE) FROM THE LAGOMORPH *LEPUS TOLAI* IN MONGOLIA

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ABSTRACT: In 1999, a single specimen of the Tolai hare, *Lepus tolai* Pallas, 1778, from the Gobi region of Mongolia was examined and had a new species of eimerian parasite in its intestinal contents. *Eimeria gobiensis* n. sp. is relatively large; it possesses 2 oocyst walls and a very well-developed oocyst residuum. Oocysts of the new species possess a thick wall with a double layer, a massive 3-layered micropyle, and are ellipsoidal, with average length and width of the oocyst of $38.6 \times 24.2 \mu\text{m}$, respectively. The range in measurements of these oocysts extends from 27.3 to 49.2 μm in length by 18.8 to 32.5 μm in width, with a length/width ratio = 1.6; the oocyst residuum is composed of a sub-spheroidal mass of small granules with an average size of $12.0 \times 11.0 \mu\text{m}$; sporocysts are ovoid with an average length \times width of $15.0 \times 7.7 \mu\text{m}$, respectively, and a range in length extending from 9.2 to 21.0 μm by 5.0 to 12.0 μm in width. In addition, each sporozoite has a large, medial, refractile body with an average size of $6.0 \times 5.0 \mu\text{m}$.

During a preliminary survey of mammalian parasite biodiversity from central Mongolia in the summer of 1999, approximately 40 species of mammals were collected from several different habitat types, representing 15 different collecting localities. From this work, 1 specimen of the Tolai hare *Lepus tolai* Pallas, 1778 (a female) was collected in the vicinity of Ulziyt Uul (see below) in the Gobi desert and examined for parasites. Tinnin et al. (2002) provided a detailed description of the habitat and a list of mammals collected from the locality. Here, we describe the coccidia recovered from this host as a presumptive new species of *Eimeria*.

MATERIALS AND METHODS

During our work in 1999, all small mammals were caught using pitfall or Sherman live traps, while large mammals, including this hare, were shot. The results of the collection and identification of the mammals themselves have been published (Tinnin et al., 2002). Specimens were examined for parasites by the senior author in the field promptly after capture, following the methods outlined in Gardner (1996). Organs were examined for the presence of metazoan parasites; fecal pellets were removed from the lower bowel of each animal and preserved in 15 ml Wheaton Snap Cap vials (available through Fisher Scientific, Pittsburgh, Pennsylvania) containing about 5 ml of 2% aqueous (w/v) potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution (Gardner, 1996).

Upon return from Mongolia, samples were refrigerated at approximately 2 C until they were studied. Oocysts found in the samples showed various stages of development, but most were completely sporulated. Close observation via standard differential interference contrast light microscopy at $\times 40$ and higher allowed us to distinguish a distinct oocyst morphotype in our samples. Oocysts were isolated and photographed as described by Duszynski and Wilber (1997). Our oocysts were measured using an integrated computerized system and photographed with a Zeiss Axiophot (Zeiss USA, <http://www.zeiss.com>), using Normarski-interference with a $\times 63$ objective oil immersion lens. In accordance with the guidelines suggested by Bandoni and Duszynski (1988), we submitted photomicrographs of the new eimerian to the Harold W. Manter Laboratory of Parasitology phototype collection, University of Nebraska–Lincoln, Lincoln, Nebraska; skin, skull, skeleton, and tissues of the symbiotype host for the new eimeriid species are preserved in the Mammal Division, Museum of Southwestern Biology, the University of New Mexico, Albuquerque, New Mexico. Abbreviations follow those of

Wilber et al. (1998): length (L), width (W), micropyle (M), oocyst residuum (OR), polar granule (PG), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), sporocyst residuum (SR), and refractile body (RB), but we use SZ for sporozoites. Measurements given include n = number of characters measured, followed by average length and width, standard deviation (SD), then range of length and width in parentheses. All measurements are in μm . Comparative measurements are presented in Table I.

DESCRIPTION

Eimeria gobiensis n. sp.

(Figs. 1–10)

Diagnosis: Sporulated oocysts ellipsoidal and elongate (n = 62) 38.6×24.2 (SD = 3.6×2.2) (27.3–49.2 \times 18.8–32.5) with L/W ratio of 1.60; wall (n = 62) 2.1 (SD = 0.5) (1.0–3.4) of uneven thickness composed of 2 layers: outer wall pale blue to transparent, smooth, $\sim 3/4$ of total thickness; inner layer yellow; PG absent, but M and OR are clearly present. The oocyst wall thickens near the margin of the prominent M. Micropyle is triple layered and very thick. The OR (n = 50) is 12.1×10.8 (SD = 1.5×1.6) (10.3–18.4 \times 9.0–17.3); sporocysts (n = 97) ovoid, tapered at 1 end, 15.0×7.7 (SD = 1.9×1.0) (9.2–20.9 \times 4.9–11.7); SR absent; SB present at tapered end, but SSB and PSB are both absent; each SZ with 1, large posterior RB (n = 193) 6.1×4.8 (SD = 1.1×1.0) (3.9–11.0 \times 2.6–8.0). Age of oocysts at time of study, calculated from time of collection to the date of isolation, was 3,343 days.

Taxonomic summary

Symbiotype host: *Lepus tolai* Pallas, 1778, Museum of Southwestern Biology, Division of Mammals, No. 94357, NK 100752 (female, approximately 2 yr of age) (see Frey et al., 1992).

Type locality: Vicinity of Ulziyt Uul, Mongolia, 1,640 m altitude (44°41'09" N, 102°00'57" E).

Prevalence: One of 1 (100%) *Lepus tolai*.

Site of infection: Unknown; oocysts recovered from feces.

Material deposited: Syntypes (=phototypes, see Bandoni and Duszynski, 1988) of sporulated oocysts, HWML Coll. No. 49155.

Etymology: The name "*Eimeria gobiensis*" is derived from the locality in which this specimen's symbiotype host was collected; "*gobiensis*" is indicative of the Gobi desert region of Mongolia, meaning "of the gobi."

Remarks

Oocysts of this eimerian do not resemble those from any species previously described from leporids of Eurasia. Specifically, *E. gobiensis* can be distinguished from all similar species described from the Leporidae by the following: from *E. hungarica* Pellérdy, 1956; *E. leporis* Nieschulz, 1923; *E. punjabensis* Gill & Ray, 1960; *E. robertsoni* Madsen, 1938; *E. ruficaudatus* Gill & Ray, 1960; *E. septentrionalis* Yakimoff et al., 1936; and *E. stefanskii* Pastuszko, 1961, in having 2 distinct oocyst wall layers. *Eimeria gobiensis* also has a well-developed OR, whereas *E. campania*

Received 23 April 2009; revised 22 July 2009; accepted 23 July 2009.

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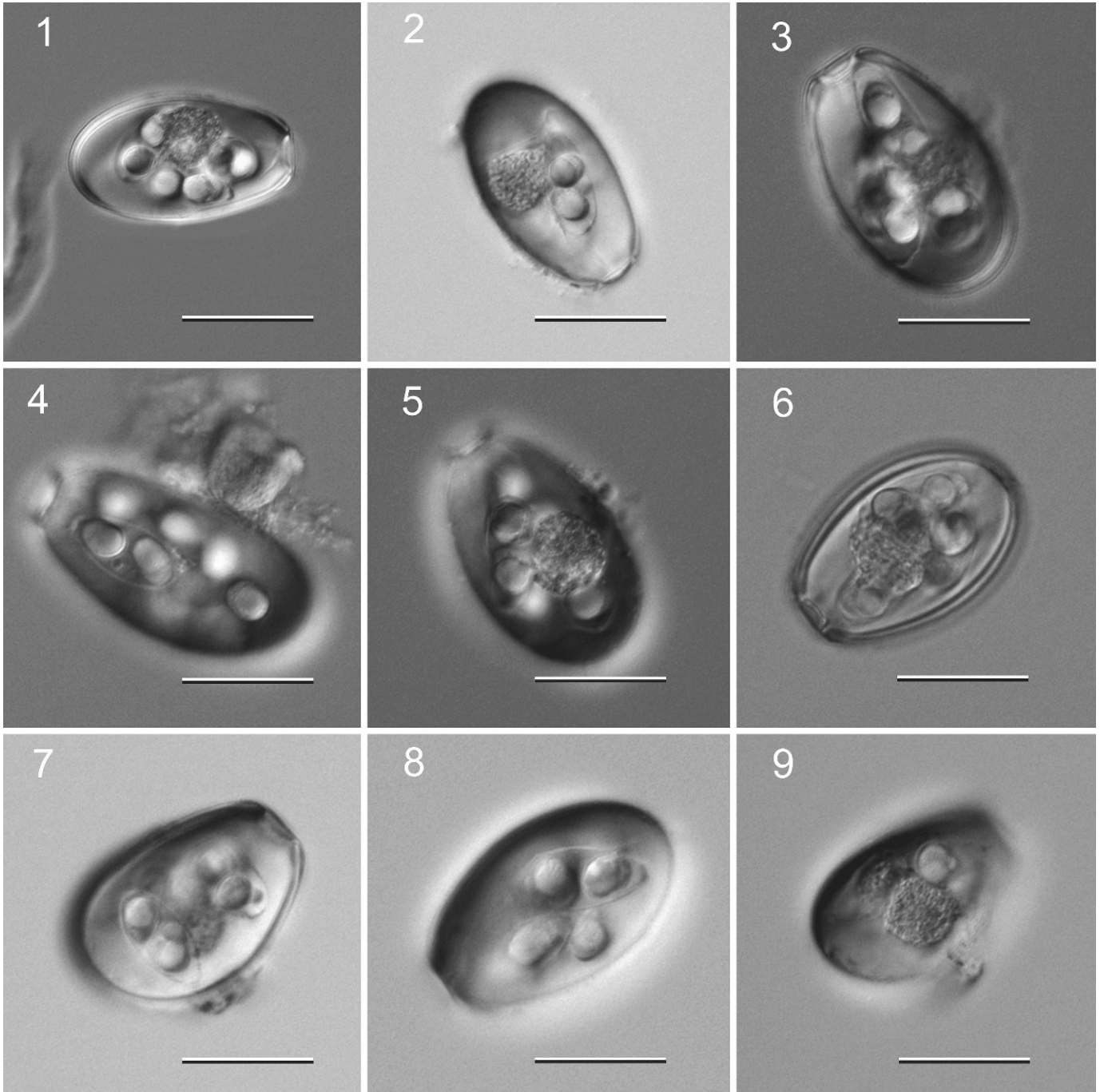
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DOI: 10.1645/GE-2137.1

TABLE I. Comparison of mensural and qualitative data of relevant species of *Eimeria* infecting members of the Family Leporidae.

Source	Species name	Host	Oocyst size	Oocyst residuum	Oocyst wall	Sporocysts	Sporozoites
Present study	<i>Eimeria gobiensis</i>	<i>Lepus tolai</i>	38.6 × 24.2 μm	Yes, 12.1 × 10.8 μm	2 Layers—2.1 μm, uneven thickness; outer thin & light yellow, inner thick, darker	15.0 × 7.7 μm, Elongate ovoid with slight tapering at one end	Head to tail orientation, w/ refractile body at larger end; 6.1 × 4.8 μm
Carvalho, 1943	<i>Eimeria campania</i>	<i>Lepus europaeus</i>	35–45 × 22–27 μm	No	2 Layers—outer very thin & brown; inner thick, colorless	18 × 9 μm	Lie head to tail, w/ globule at wider end
Pellérdy, 1956	<i>Eimeria europaea</i>	<i>L. europaeus</i>	26–34 × 15–20 μm	Yes	2 Layers—outer pale, inner dark, both gray-yellow	9 × 6 μm, Possible residuum	Lie head to tail, w/ globule at wider end
Pellérdy, 1956	<i>Eimeria hungarica</i>	<i>L. europaeus</i> ,	12–15 × 11–14 μm	Unclear	Single thin layer—smooth and colorless	“Stocky,” no residuum	Contain large, clear globule
Nieschulz, 1923	<i>Eimeria leporis</i>	<i>Lepus ruficaudatus</i> <i>L. tolai</i>	26–38 × 13–20 μm	Yes	Single thin layer—smooth and colorless/pale yellow	Elongate ovoid, residuum present	Lie head to tail w/ globule at wider end
Gill & Ray, 1960	<i>Eimeria punjabensis</i>	<i>L. ruficaudatus</i>	20–24 × 19–23 μm	Yes	Single layer—light yellow to salmon tinted	12.5 × 8.5 μm	Lie head to tail, w/ globule at wider end
Madsen, 1938	<i>Eimeria robertsoni</i>	<i>L. europaeus</i> , <i>L. ruficaudatus</i>	34–52 × 23–33 μm	Yes	Single layer—yellowish to yellowish-brown	Spindle-shaped, no residuum	Lie head to tail, w/ globule at wider end
Gill & Ray, 1960	<i>Eimeria ruficaudatus</i>	<i>L. ruficaudatus</i>	23–35 × 12–15 μm	Yes	Single thin layer—yellowish-pink	Ovoid, residuum present	Lie head to tail, w/ globule at wider end
Yakimoff et. al, 1936	<i>Eimeria septentrionalis</i>	<i>Lepus townsendi</i> <i>campanius</i>	21–32 × 17–27 μm	No	Single smooth layer ~1 μm thick	Ovoid to sausage-shaped, 12–16 × 6–8 μm	Lie head to tail, w/ globule at wider end
Pastuszko, 1961	<i>Eimeria stefanski</i>	<i>L. europaeus</i>	59–68 × 32–37 μm	Yes	Single layer—yellow to dark brown	15 μm Long, ovoid, no residuum	Lie head to tail, w/ globule at wider end
Carvalho, 1943 emend. Pellérdy, 1956	<i>Eimeria townsendii</i>	<i>L. europaeus</i>	37–44 × 25–31 μm	No	2 Layers—smooth—inner yellow, outer dark brown	Spindle-shaped, 17 × 10 μm, residuum present	Lie head to tail, w/ globule at wider end



FIGURES 1–9. Photomicrographs of sporulated oocysts of coccidia recovered from the feces of *Lepus tolai* Pallas, 1778. *Eimeria gobiensis* n. sp. Note prominent oocyst residuum (1, 2, 5, 6, 9), thick, bi-layered oocyst wall (1, 6), long, tapered sporocysts, and head to tail orientation of sporozoites with large refractile bodies (1, 4, 5, 7, 8), and prominent triple-layered micropyle (1, 3, 6). Scale bar = 25 μ m.

Carvalho, 1943, and *E. townsendii* (Pellérdy, 1956) have none. Finally, *E. gobiensis* can be distinguished from *E. europaea* Pellérdy, 1956 based on the size of the oocyst. In *E. gobiensis*, the average oocyst size is 38.6×24.2 vs. $26\text{--}34 \times 15\text{--}20$, respectively.

DISCUSSION

Species of *Eimeria* are common in lagomorphs globally; consequently, the presence of coccidia in *L. tolai* from Mongolia is not surprising. While the locality from which the parasite

specimens were obtained is superficially similar in an ecological sense to regions in central North America, their inherent isolation from these populations presents new opportunities for study. Although beginning work has been conducted to document the biodiversity of mammals and their parasites in Mongolia and central Asia, the actual extent of the literature and studies available is woefully deficient compared to other areas of the world. Research institutes from Mongolia, Russia, and Germany have made progress in the latter half of the 20th century in

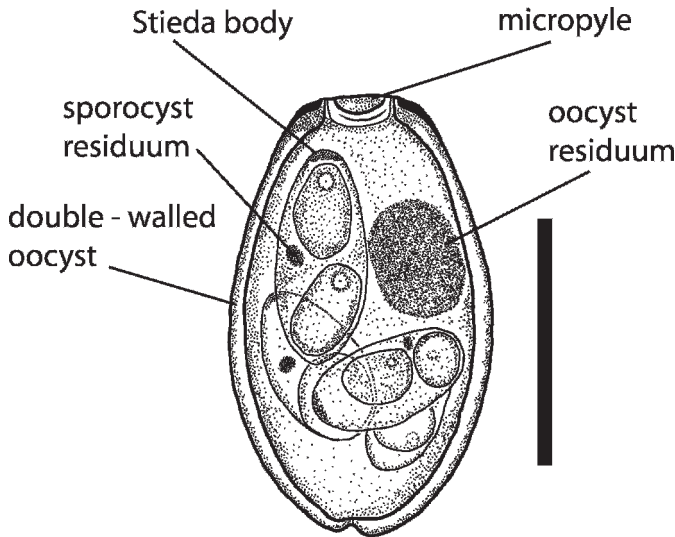


FIGURE 10. Line drawing of *Eimeria gobiensis* n. sp. with major structures in the oocyst labeled. Scale bar = 25 μ m.

documenting the mammalian fauna of Mongolia, but there are still several areas, i.e., parasitology, that are very poorly known.

Since this report is based on a very short field expedition with limited time for collecting a larger series of mammals, we expect that results from our continuing work in Mongolia will provide more data on prevalence and diversity of eimerian species in these mammals.

ACKNOWLEDGMENTS

This work was supported by NSF grants DEB-0717214, DEI-0646356, DBI-9631295, and DBI-9411976. Additionally, we thank Gabor Racz, Ethan Jensen, and Agustin Jimenez for assistance with microscopy, photomicrography, and general support during this work.

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