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Coccidia (Apicomplexa: Eimeriidae) from *Vespertilio murinus* and *Eptesicus gobiensis* (Chiroptera: Vespertilionidae) in Mongolia and How Many Species of Coccidia Occur in Bats?

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Coccidia (Apicomplexa: Eimeriidae) from *Vespertilio murinus* and *Eptesicus gobiensis* (Chiroptera: Vespertilionidae) in Mongolia and how many species of Coccidia occur in bats?

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Abstract

An examination of the feces from 28 bats collected in 1999 and from 12 bats collected in 2009, all from Mongolia, revealed the presence of two new species of coccidian parasites of the genus *Eimeria*. Bats representing two species assigned to different genera were studied including: *Vespertillio murinus* and *Eptesicus gobiensis.* Oocysts of *Eimeria stubbei* n. sp. from *V. murinus* collected in 1999 are ellipsoid, average length and width of 22.4 x 18.7 µm, with a 1.5 µm thick double layered wall and a single polar granule. Sporocysts of this species are ovoid, 10.0 x 7.2 µm in length and width, with a Steida body and a sporocyst residuum consisting of 2–3 globules. Oocysts of *Eimeria samiyai* n. sp., from *E. gobiensis* collected in 2009 are ellipsoid, 26.3 x 19.9 µm in average length and width, with a 1.6 µm thick double layered wall and a single polar granule. Their sporocysts are ovoidal, with a length and width of 11.9 x 7.7 µm with a prominent Steida body and a sporocyst residuum.

Key words: *Vespertilionidae, Vespertilio murinus, Eptesicus gobiensis*, Coccidia, *Eimeria stubbei, Eimeria samiyai*, Gobi desert, Mongolia oocyst

Introduction

Coccidia that occur in bats of the family Vespertilionidae have not been studied intensively, although DUSZYNSKI (2002) reported two species of *Eimeria* from *V. murinus* LINNAEUS, 1758 collected in Azerbaijan, including, *Eimeria vespertilii* MUSAEV and VEISOV, 1961; and *Eimeria zakiraca* MUSEAV, 1967. Up to the present time, approximately 258 individuals of *Eptesicus fuscus* (PALISOT DE BEAUVOIS, 1796) (Chiroptera: Vespertilionidae) from Baja California Sur, Mexico and the United States (New Mexico, California, Wyoming, South Carolina, Arkansas, and Alabama) have been examined for coccidia (DUSZYNSKI 2002, DUSZYNSKI et al. 1988, DUSZYNSKI et al. 1999, McALLISTER & UPTON 2009, SCOTT & DUSZYNSKI 1997, SEVILLE & GRUVER 2004). Of those, only a single individual bat collected in New Mexico was shown to be infected with a species of *Eimeria*, but it was judged that there were too few oocysts present to adequately describe the species (DUSZYNSKI et al. 1999). In addition, four individuals from a captive colony of *E. fuscus* in Alabama were reported to be infected with oocysts resembling *Isospora* with foci of infection in their kidneys. The results of the study from Alabama was presented as a poster at the American Society of Parasitologists annual meeting in 2000; however, to our knowledge, these results have not been published (DUSZYNSKI 2002, SUNDERMAN et al. 2000). Of the other species of *Eptesicus*, only a single individual of *E. brasiliensis* (DESMAREST, 1819) from Bolivia that was collected during the field component of the Bolivian Mammal Parasite Survey, was examined for the presence of coccidia, and this individual was shown to be uninfected (DUSZYNSKI 2002, SCOTT & DUSZYNSKI 1997, see also ANDERSON 1997).

Species of bats in the genus *Vespertilio* LINNAEUS, 1758 have a wide geographic distribution in the Palearctic (SIMMONS 2005). At the present time, only two species are recognized in this genus, including *V. murinus*, and *V. sinensis* (PETERS, 1880) (see SIMMONS 2005, WALKER 1968). *Vespertilio murinus* occurs in suitable habitat throughout the Palearctic, ranging from Britain as far north as Norway east through Russia and south through Afghanistan, Mongolia, and China (TINNIN et al. 2002). *Vespertilio sinensis* has a more limited distribution in the eastern Palearctic

ranging from a northernmost limit in Siberia, south and west through China, Mongolia, Korea, Taiwan and Japan (SIMMONS 2005, WALKER 1968). Within Mongolia, *V. murinus* has been collected from several geographically disparate localities, while specimens of *V. sinensis* have been reported from only from 3 localities representing riparian habitat in the grassland-steppe biome at the far eastern border of the country (BATSAIKHAN et al. 2010, TINNIN et al. 2002).

Species of the genus *Eptesicus* RAFINESQUE, 1820 occur world-wide, with the exception of islands in the south Pacific Ocean. Evidently an excellent long distance disperser, these bats have been collected from many larger oceanic islands such as Hawaii, islands in the the Carribean, and from Madagascar. Approximately 23 species of *Eptesicus* are currently recognized, five of which occur in central Asia (SIMMONS 2005). Three species of *Eptesicus* have been recorded from Mongolia including: *E. gobiensis* BOBRINSKII, 1926, *E. nilssonii (*KEYSERLING and BLASIUS, 1839), and *E. serotinus* (SCHREBER, 1774) (see BATSAIKHAN et al. 2010, TINNIN et al. 2002). *Eptesicus gobiensis* is known primarily from southern desert and semi-desert regions of Mongolia although there are collection records from more mesic northern steppe zones as well (BATSAIKHAN et al. 2010, DOLCH et al. 2007, TINNIN et al. 2002).

In central Asia, TINNIN et al. (2002) reported that 62 bats were collected in 1999 from central Mongolia during a joint expedition from the Museum of Southwestern Biology, the Manter Laboratory of Parasitology, and the National University of Mongolia. Of the bats collected from several localities, 28 were examined for coccidia, and a survey of the south central Gobi in the summer of 2009 yielded another 32 bats of various species of which 12 were examined for coccidia. A single individual from each survey year was found to be infected with coccidia of the genus *Eimeria*. This report documents these findings and describes two new species of *Eimeria*.

Materials and Methods

Bats were captured in the field using mist nets and by shotgun. Specimens were necropsied in the field promptly after capture following the methods outlined in GARDNER (1996) and GARDNER & JIMÉNEZ-RUIZ (2009). Fecal material from the lower bowel of each animal examined was preserved in 15 ml Wheaton Snap Cap vials containing approximately 5 ml of 2.0 % aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) solution. After transport back to the laboratory (at ambient temperatures) samples were refrigerated at 2º C until study. Aliquots of each sample were later isolated using coverslip flotation as described by DUSZYNSKI & WILBER (1997). The samples in which oocysts were found were examined multiple times in order to obtain the maximum amount of measurement data, and photographs were taken of each oocyst found during each examination. Overall, 14 oocysts were found, photographed and measured using a Jenaval compound microscope with a Pixera™ PVC100C camera and SigmaScan Pro 5 software. More than 10 oocysts were found, photographed and measured using a Zeiss Axioplan 2 microscope with integrated Zeiss computerized measurement system.

Methods defining characters required to validate species of *Eimeria* and character abbreviations used in this paper follow those set by WILBER et al. (1998). Characters of the oocyst include: length (L), width (W), length to width ratio (L/W), micropyle (M), oocyst residuum (OR) and polar granule (PG). Characters of the sporocyst include: length (L), width (W), length to width ratio (L/W), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), and refractile bodies (RB). All measurements are given in µm. Measurements include presence or absence of characters, number of characters measured (n), average length or width ± standard deviation, range in parentheses, and qualitative observations.

Following DUSZYNSKI & WILBER (1997), photomicrographs were submitted to the Harold W. Manter Laboratory of Parasitology phototype collection, University of Nebraska State Museum, University of Nebraska-Lincoln, and all symbiotype host material are deposited in the Mammal Division of the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico.

Results

Coccidia were found in three of 28 bats examined from our work in Mongolia in 1999. However, in a single specimen of *V. murinus*, only a single, unsporulated oocyst was found. In another individual of *Myotis brandti* (EVERSMANN, 1845), seven sporulated oocysts were found but they had degenerated and could not be identified.

The following descriptions are based on sporulated oocysts found in one individual of *V. murinus* from the 10 specimens examined in 1999 and from one individual of *E. gobiensis* from the 12 individuals examined of those collected in 2009.

Descriptions

***Eimeria stubbei* n. sp.**

Diagnosis (Figs. 1-4)*:*

Sporulated oocysts are ellipsoidal (n = 9) 22.4 ± 1.9 x 18.7 ± 1.3 (18.8-26.4 x 15.9-23.1) with L/W ratio of 1.8; wall (n = 9) 1.5 ± 0.2 (1.2-2) of uneven thickness consisting of a rough outer layer (~1/3 thickness of wall) and smooth inner layer; single polar granule present but oocyst residuum and micropyle absent; sporocysts (n = 38) 10.0 ± 1.2 x 7.2 ± 0.6 (7.4-11.4 x 6.2-8.0), sporocyst residuum and Stieda body present but sub Stieda body and parastieda body absent; refractile bodies absent; sporocyst residuum consists of 2–4 globules about 1.9 in diameter; Stieda body approximately 1.5 deep x 2 wide. Oocysts were observed 2,900 days after the host was collected.

Taxonomic summary

Symbiotype:	*Vespertilio murinus* LINNAEUS, 1758, Museum of Southwestern Biology, Division of Mammals, MSB94099, NK100659 (adult female) (see FREY et al. 1992).
Type locality:	Mongolia, Övörkhangay Aymag, Ulaan Tsutgalan; 1,850 m altitude, (46˚47’13” N, 101˚57’47” E).
Prevalence:	One of 10 (10 %) *V. murinus* at the type locality were infected.
Site of infection in host:	Unknown, oocysts recovered from feces.
Material deposited:	Syntypes (= phototypes, see BANDONI & DUSZYNSKI 1988) of sporulated oocysts, HWML Coll. No. 49568.
Etymology:	The species is named *“Eimeria stubbei”* in honor of Dr. Michael STUBBE, (Professor Emeritus from Martin-Luther University, Halle, Wittenberg) who was personally responsible for maintaining the philosophical and physical presence for the long-term project on discovery, exploration, and documentation of biodiversity in Mongolia from 1962–2012.

Differential diagnosis

Oocysts of *E. stubbei* are structurally distinct from those of other species of *Eimeria* known to infect bats of the genus *Vespertilio* and *E. stubbei* can be distinguished from *E. vespertillii* based on the shape of the oocyst, which is ellipsoid versus subspheroid; smaller size, L x W: 22.4 x 18.7 versus 25.0 x 21.0; lack of an oocyst residuum, and a rough rather than smooth outer wall.

Eimeria stubbei can be distinguished from *E. zakirica* by the shape of the oocyst which is ellipsoid versus subspheroid, smaller size, L x W: 22.4 x 18.7 versus 25.0 x 22.5, presence of a Stieda body, double instead of single walled oocyst, and a rough rather than smooth outer wall of the oocyst.

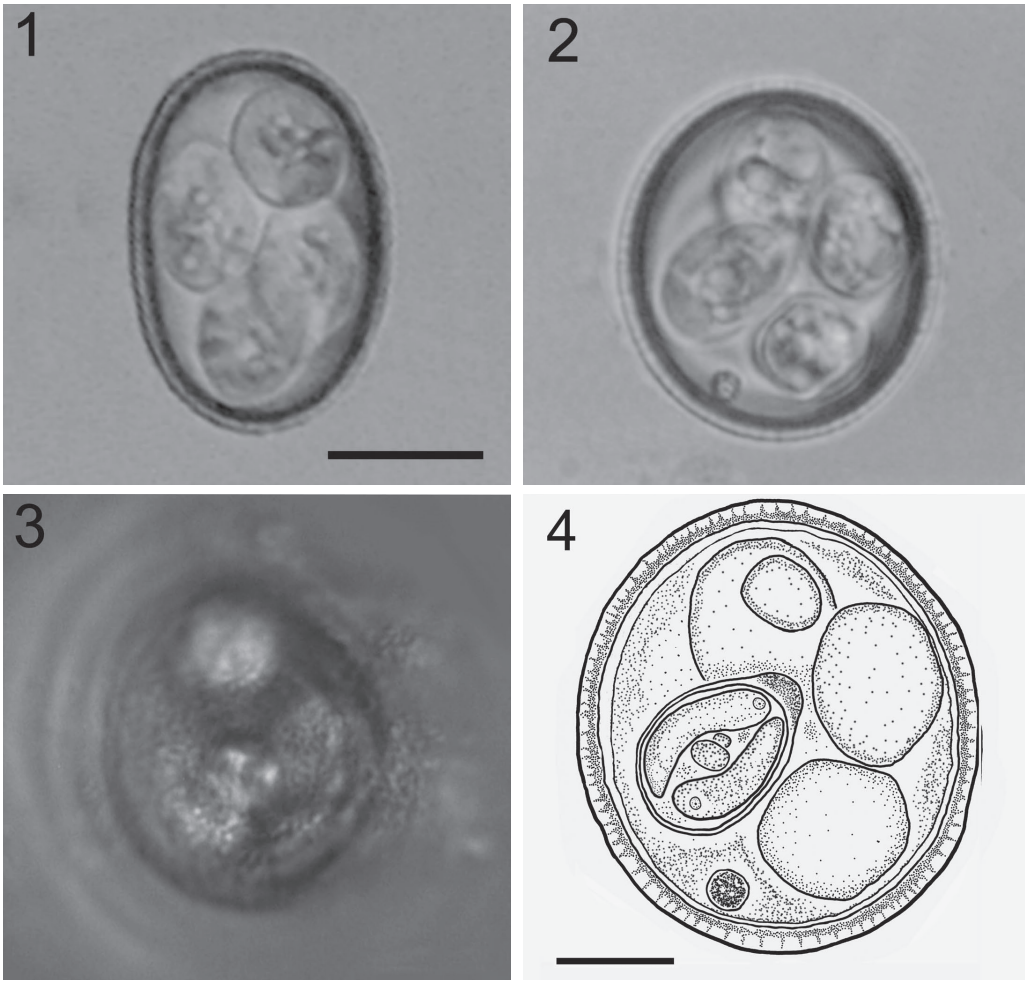

Fig. 1-3: Photomicrographs of sporulated oocysts of coccidia recovered from the feces of *Vespertilio murinus*. *Eimeria stubbei* n. sp. **1-3**. Note thick, bilayered wall (**2**) with rough outer layer (**3**) and absence of oocyst residuum (**1, 2**).

Fig. 4: Drawing of *Eimeria stubbei* n. sp.; scale bar = 10 µm.

***Eimeria samiyai* n. sp.**

Diagnosis (Figs. 4-6, 8)*:*

Sporulated oocysts are ellipsoidal (n = 3) 26.3 ± 1.3 x 19.9 ± 0.7 (25.2-27.7 x 19.1-20.4) with L/W ratio of 1.3; wall (n = 3) 1.6 ± 0.1 (1.5-1.7) of uneven thickness consisting of a somewhat dimpled outer layer (slightly more than 1/2 thickness of wall) and smooth inner layer; micropyle approx. 5 in width and approx. 2 in depth, without cap; single polar granule approx. 2 in diameter; oocyst residuum absent; sporocysts (n = 12) 11.3 x 8.1 (9.8-13.3 ± 1.6 x 6.7-8.6 ± 0.5), sporocyst residuum, Stieda body and 1 or two refractile bodies present but sub Stieda body and para Stieda body absent; sporocyst residuum granular approximately 6 in diameter; Stieda body prominent and nipple-like ~3.4 deep and ~4.9 wide, RB 4 in diameter. Oocysts studied approximately 242 days after collection in field.

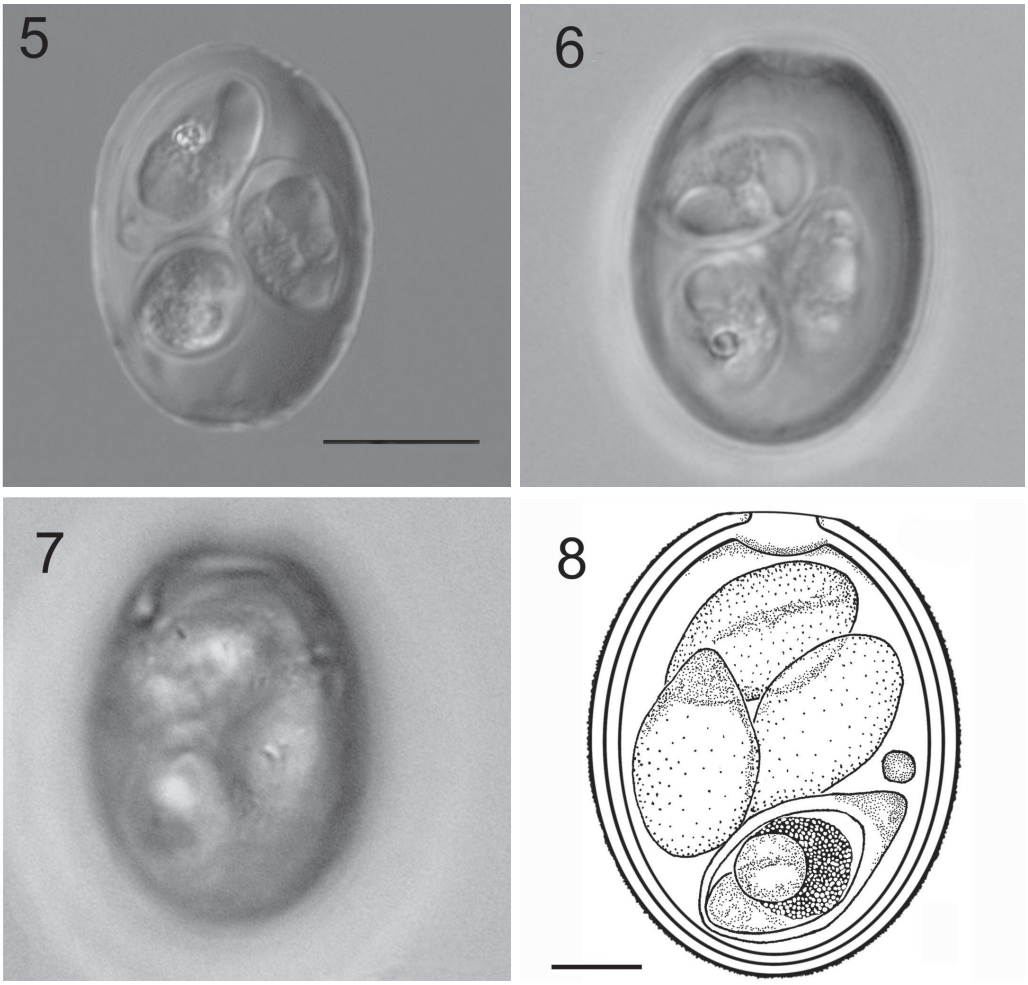

Fig. 5-7: Photomicrographs of sporulated oocysts of coccidian recovered from the feces of *Eptesicus gobiensis*. *Eimeria samiyai* n. sp. **5-7**. Note bilayered wall (**5**) with somewhat dimpled outer layer (**6**), micropyle (**5**), lack of oocyst residuum (**4, 5**) consolidated, granular sporocyst residuum (**4**) and large refractile bodies (**5**). Scale bar = 10 µm.

Fig. 8: Line drawing of *Eimeria samiyai* n. sp.; scale bar = 10 µm.

Taxonomic summary

Symbiotype:	*Eptesicus gobiensis* BOBRINSKI, 1926, Museum of Southwestern Biology, Division of Mammals, No. 215037, NK 166628 (adult male) (see FREY et al. 1992).
Type locality:	Mongolia, Tsagaan Ovoo Uul, Gobi Gurvan Saikhan National Park; 2,308 m altitude, (43˚37'4.5" N, 103˚45'16.4" E).
Prevalence:	One of six (17 %) *E. gobiensis* from the type locality were infected.
Site of infection:	Unknown, oocysts recovered from feces.
Material deposited:	Syntypes (= phototypes, see BANDONI & DUSZYNSKI, 1988) of sporulated oocysts, HWML Coll. No. 49569.

Etymology: The species is named *Eimeria samiyai* after Professor Ravchig Samiya, an ardent and dedicated proponent of the long-term and intensive study and conservation of the Biodiversity of Mongolia.

Differential diagnosis

Eimeria samiyai can be distinguished from *E. verspertilii* by having an oocyst with an ellipsoid versus subspheroidal shape, absence of an oocyst residuum, presence of a micropyle, presence of a refractile body, and noticeably larger sporocysts, L x W: 11.3 x 8.1 versus 9.0 x 5.0, respectively. In addition *E. samiyai* has a sporocyst residuum composed of a consolidated granular mass rather than a few scattered granules as in E. verpertilioni. In addition *E. samiyai* can be separated from *E. zakirica* by the shape of the oocyst which is ellipsoid versus subspheroidal, the presence of a Stieda body and refractile body, and a thick, two-layered wall instead of a thinner single-layered wall as in *E. zakirica*.

Two species of coccidia (*Eimeria nyctali*, GOTTSCHALK, 1974; and *Eimeria vejsovi* CERNA, 1976) have been described from individualsof *Nyctalus noctula* (SCHREBER, 1774) from europe, a species of bat that also occurs in Mongolia (DUSZYNSKI 2002, TINNIN et al. 2002). *Eimeria samiyai* n. sp. differs from *E. nyctali* by having an ellipsoid versus subspheroidal shape, a rough versus smooth wall, by possessing a polar granule, a micropyle, and a Stieda body and a refractile body. *Eimeria samiyai* n. sp. differs from *E. vesjovi* n. sp. by having an ellipsoid versus subspheroidal shape, a rough versus smooth wall, by being much larger (6 versus 3.5) in diameter, by having a sporocyst residuum that is not membrane bound and by the presence of a sporocyst residuum.

Discussion

This paper represents the first report of parasites of the genus *Eimeria* from species of *Eptesicus* from the Palearctic region. The only other report of *Eimeria* from any bats of the genus *Eptesicus* was an undescribed form from *E. fuscus* in North America (DUSZYNSKI et al. 1999). Although commonly reported in rodents (LAMBERT, GARDNER & DUSZYNSKI 1988; GARDNER & DUSZYNSKI 1990), infections of several species of bats by a single species of *Eimeria* have not been commonly reported in the literature. Interestingly, the oocysts of *E. samiyai* are structurally distinct from those of other species of *Eimeria* known to infect bats of the family Vespertilionidae. Additional work remains to be conducted to observe whether these may potentially co-occur with *Eptesicus gobiensis* either in ecological or geographic space.

Neither *E. verpertilii* nor *E. zakirica* has been observed since their original descriptions (DUSZYNSKI 2002), nor have there been recorded observations of either species since the publication of that review. Therefore, these new records are not only the first new species but the first new observations of *Eimeria* in *Vespertilio* in over 30 years. This also represents only the second occurrence and the first described species of *Eimeria* from members of the genus *Eptesicus*, a fairly large group with representatives known from world wide.

How many species of Eimeria occur in bats?

The few species of coccidia reported from bats from the Gobi and world-wide most likely reflects the paucity of investigations into the coccidian parasites of bats in general. DUSZYNSKI's (2002) estimated that from only 2,119 individual bats examined for coccidia worldwide, (from only 86 species of the over 900 species of bats currently recognized) the vast majority were species collected in the Nearctic and Neotropical regions by DUSZYNSKI and his colleagues (DUSZYNSKI 2002).

Interestingly, the species most often examined for coccidia has been *E. fuscus* from the Nearctic. However, the prevalence within those species of bats examined averaged 11 % in total, and

DUSZYNSKI estimated that as many as 1800 species of coccidia may be present and awaiting discovery in the bats of the world. We consider this a low estimate as we expect that some bats with great geographic ranges will harbor more than two species of coccidian, so we would estimate, at the low end about 1800 to a high of around 2700 species of *Eimeria* in bats world wide.

This report is the second in our series on the coccidia of Mongolian mammals (GARDNER et al. 2009). It is our hope that our ongoing work in Mongolia will continue to provide additional data concerning the biodiversity of species of parasites, not only in bats, but also of the other members of the mammal fauna of this country.

This paper was a contribution to the volume assembled by Professor M. STUBBE celebrating 50 years of field expeditions by joint Mongolian – German teams throughout Mongolia.

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