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New species of *Eimeria* (Apicomplexa: Eimeriidae) from *Ochotona hyperborea* and *Ochotona pallasii* (Lagomorpha, Ochotonidae) in Mongolia

D.S. Tinnin, E.T. Jensen, N. Batsaikhan, S. Ganzorig & S.L. Gardner

Abstract

An examination of the feces from 8 pikas collected in 1999 and from 17 pikas collected in 2009 in Mongolia revealed the presence of 3 new eimerian species. Four of the 5 species of pikas present in Mongolia were studied including: *Ochotona alpina*, *O. dauurica*, *O. pallasii*, and *O. hyperborea*. Oocysts of *Eimeria dunnumi* n. sp. from *O. hyperborea* collected in 1999 are ellipsoid, average length and width of 31.4 x 20.8 µm, with a 1.4 µm thick double layered wall, lacking a micropyle, oocyst residuum, and polar granule. Sporocysts of this species are ellipsoid, 12.8 x 8.1 µm in length and width, with a steida body and a compact sporocyst residuum. Oocysts of *Eimeria burti* n. sp., from *O. pallasii* collected in 2009 are ovoid, 26.3 x 21.1 µm in average length and width, with a 1.6 µm thick double layered wall with an oocyst residuum. Their sporocysts are ellipsoidal, with a length and width of 11.4 x 7.8 µm with a prominent steida body. Oocysts of *Eimeria salazarbravoii* n. sp., from *O. pallasii* collected in 2009 are ovoidal, 26.6 x 20.5 µm in average length and width, with a 1.6 µm thick double layered wall, with a micropyle. Their sporocysts are ellipsoidal, with a length and width of 11.6 x 7.6 µm with a prominent Steida body and a compact sporocyst residuum.

Species of *Ochotona*, pikas, are found in habitats ranging from semi-desert to taiga and high mountains across the Holarctic. The majority of the 30 currently recognized species occur in Asia, with only 2 species occurring in North America (HOFFMANN & SMITH 2005). Five of the Asian representatives of this genus occur in Mongolia. *Ochotona hyperborea* (PALLAS, 1811), the northern pika, has a broad distribution across Asia, from the Ural Mountains to the Pacific and south into northern Mongolia and China. In Mongolia they are common in the talus slopes and mountain steppe habitats of the northern part of the country. PALLAS' pika, *Ochotona pallasii* (GRAY, 1867), has a disjunct distribution, with populations of this species ranging from Kazakhstan east through the Altai Mountains, across Mongolia into the Tuva region of Russia, and Xinjiang and Inner Mongolia of China. This species is found in the southern region of Mongolia in the southern Khangay Mountains and along the stretch of the Gobi Altai and Mongolian Altai Mountains. The alpine pika, *Ochotona alpina* (PALLAS, 1773), is found from northwestern Afghanistan across southern Russia, northern Mongolia, and China.

In Mongolia this species ranges across the Mongolian Altai and the Khentey and Khangay Mountains. *Ochotona dauurica* (PALLAS, 1776), the Daurian pika, has a range extending east from the Altai Mountains of Russia across Tuva and Transbaikalia, and south into Mongolia and China. They occupy suitable habitat through the Mongolian and Gobi Altai Mountains as well as steppe habitats in the northern part of Mongolia. HOFFMANN's pika, *Ochotona hoffmanni* (FORMOZOV et al., 1996), has the smallest geographic distribution, occurring only in the northern Khentey Mountains and bordering areas of the Transbaikal region of Russia (TINNIN et al. 2002, HOFFMANN & SMITH 2005, BATSAIKHAN et al. 2010, TINNIN et al. 2011a).

Recent papers have addressed the apicomplexan parasites of pikas from Beringia and northern China (LYNCH et al. 2007, CAO et al. 2009). To date there have been 18 species of *Eimeria* described from 7 species of *Ochotona* from scattered localities ranging across the Holarctic from Turkmenistan to Colorado (LYNCH et al. 2007, CAO et al. 2009). There have been, however, no

reports of *Eimeria* from Mongolian pika, although we have contributed previous data from other Mongolian host species (GARDNER et al. 2009, TINNIN et al. 2011b). Herein we continue our efforts to report on Mongolian Apicomplexa represented by the description of 3 new species of *Eimeria*.

Key words: *Ochotona alpina*, *O. dauurica*, *O. pallasi*, *O. hyperborea*, oocysts of *Eimeria dunnumi*, *Eimeria burti*, *Eimeria salazarbravoi*, Mongolia, Pika

Materials and Methods

Field work in Mongolia, conducted in 1999 and 2009, resulted in the collection of fecal samples, endo- and ectoparasites, and host material from small mammals from numerous localities (TINNIN et al. 2002, GARDNER et al. 2009, TINNIN et al. 2011b). Pikas were collected by trapping in Sherman® and Tomahawk® live traps as well as with .22 cal. rifles and shotguns. Mammals were necropsied shortly after their capture following the methods of GARDNER (1996). Hosts were brushed for ectoparasites, their organs and body cavities were examined for metazoan parasites, and fecal pellets were collected from the distal end of the large intestine. Fecal samples were preserved in 5 ml of 2 % aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) in 15-ml Wheaton® Snap Cap vials. Following return from the field, vials were stored at 2° C in potassium dichromate, approximately 2785 days for samples from 1999 and 220 days for 2009 samples, until examined by the coverslip flotation method of DUSZYNSKI et al. (1982).

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. Oocysts from the 1999 collection were examined, imaged, and measured using a Jenaval® compound microscope, Pixera® PVC100C camera and SigmaScan® Pro 5 software. Those from the 2009 collection were examined and imaged using a Zeiss® Axioplan 2 integrated computerized system.

Methods and character abbreviations follow those set by WILBER et al. (1998). Characters of the oocyst considered were length (L), width (W), length to width ratio (L/W), micropyle (M), oocyst residuum (OR) and polar granule (PG). Characters of the sporocyst considered were 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). Measurements are reported in μm with the sample size (n), means, and the standard deviation and range followed in parentheses. A multi-group discriminant analysis was performed using Statistical Analysis Systems (SAS version 9.2) on five quantitative measurements of the three eimerians described in this study (ANONYMOUS 1989).

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

Results

During the field work in Mongolia in the summer of 1999, 8 individuals from 3 species (*O. alpina*, *O. dauurica* and *O. hyperborea*) from 3 localities in the north-central region of the country were examined for coccidia. Four individuals of *O. dauurica* from Ulaan Tsutgaalan, a rocky, river-canyon in steppe habitat, and 1 individual from the strictly steppe region of Khetsuugiin Ovur were uninfected. Two individuals of *O. alpina* from Ulaan Tsutgaalan were examined, 1 of whom had a few unsporulated oocysts while the other was uninfected. The remaining individual, of *O. hyperborea* from the talus fields among the mixed forest of Gorkhi-Terelj National Park, was infected with a new species described below.

In 2009, 3 individuals of *O. dauurica* and 14 individuals of *O. pallasi* from Tsaagan Ovoo Uul, a sparsely vegetated shrubby mountain in the Dund Saikhan Mountain range in the Gobi desert, were examined for coccidia. A single Pallas' pika was infected with 2 species of *Eimeria*, the descriptions for which are detailed below.

Descriptions

Eimeria dunnumi n. sp.

Diagnosis (Figs. 5, 6, 9):

Oocysts ellipsoidal, tapering slightly at opercular end ($n = 33$) 31.4×20.8 (SD = 2.1×0.7) (24.5 - 33.7×19.5 - 22.0); with a length/width ratio of 1.5; wall ($n = 33$) 1.4 (SD = 0.1) (1.4-1.6) of even thickness, outer wall smooth, green ~3/4 of total thickness; inner wall yellow; M, OR, and PG absent. Sporocysts ($n = 71$) ellipsoidal, tapering 12.8×8.1 (SD = 1.7×1.1) (8.2 - 15.6×6.2 - 12.8). SB present, but SSB and PSB both absent; compact SR a single granular mass ~4.6 in diameter. Sporozoites elongated, arranged side by side and offset; large posterior RB present. Age of oocysts at time of study, calculated from time of collection to the date of isolation, was 2,785 days.

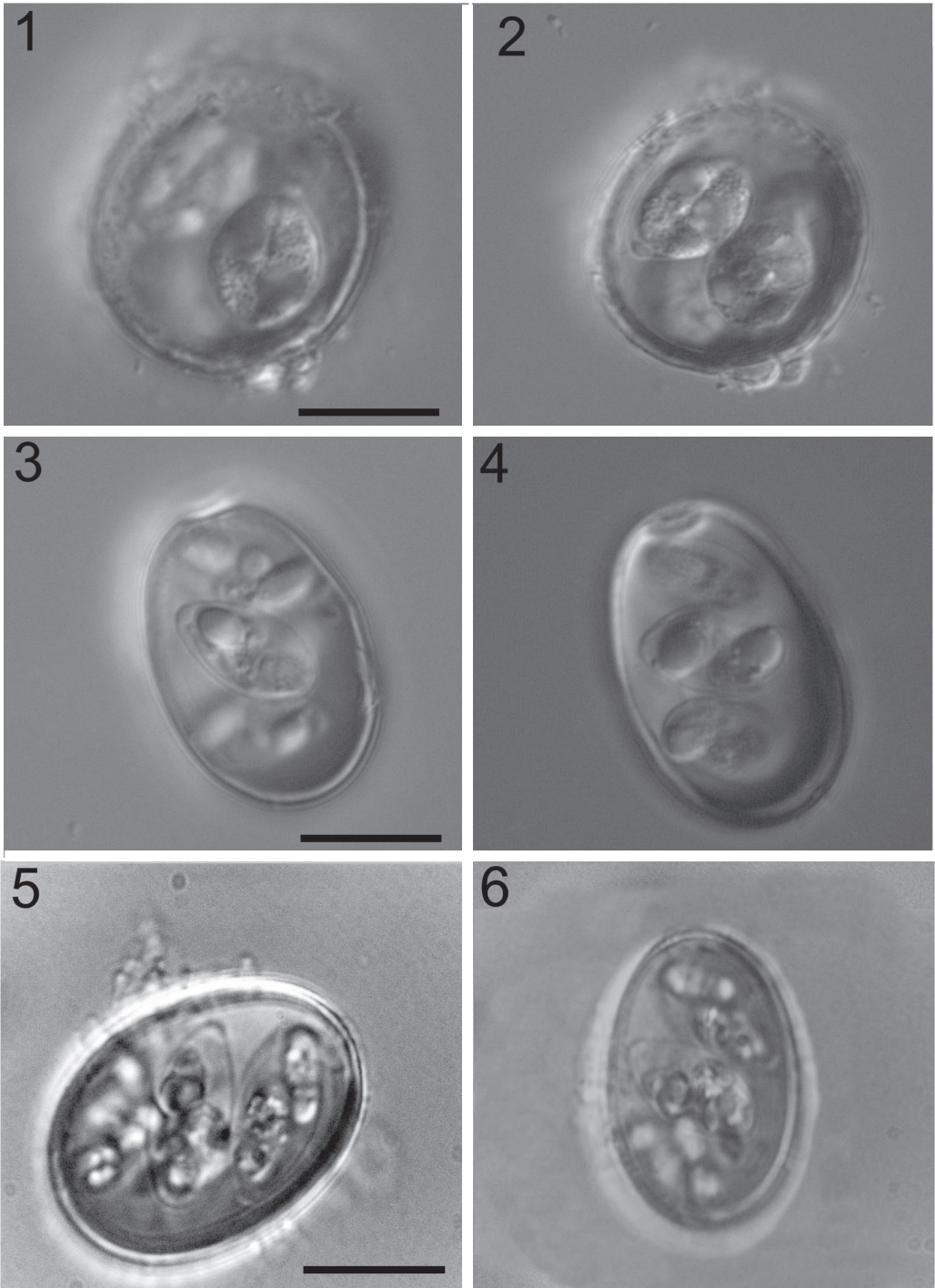
Taxonomic summary

- Symbiotype*: *Ochotona hyperborea* (PALLAS, 1811), Museum of Southwestern Biology, MSB 94354, NK 100563 (subadult female) (see FREY et al. 1992).
- Type locality*: Mongolia; TövAimag, Gorkhi-Terelj National Park, $47^{\circ}53'$ N, $107^{\circ}23'$ E, 1,800 m elevation.
- Date collected*: 13 July, 1999.
- Prevalence*: One of 1 (100 %).
- Site of infection*: Unknown, oocysts recovered from feces.
- Material deposited*: Phototypes (see BANDONI & DUSZYNSKI 1988) of sporulated oocysts, H.W. Manter Laboratory of Parasitology, HWML 49771.
- Etymology*: This species is named in honor of Dr. Jon L. Dunnum of the Museum of Southwestern Biology at the University of New Mexico, a long term supporter of our work on Mongolian biodiversity, colleague, and member of the expedition in 1999.

Remarks

Members of *E. dunnumi* are distinct in several characters from those of the other species of *Eimeria* known to infect species of *Ochotona*. In general, oocysts of *E. dunnumi* are more elongate and larger than the other species that lack a micropyle that include: *E. worleyi* LEPP et al. 1972, *E. pallasi* LEPP et al. 1972, *E. dauurica* MACHULSKII 1949, *E. banfensis* LEPP et al. 1973, and *E. princepsis* DUSZYNSKI & BRUNSON, 1973. More specifically *E. dunnumi* differs from *E. worleyi* in lacking a PG, being sub-spheroid with a length/width ratio of 1.5 versus 1.08, and being significantly larger in measurements of both the oocysts 31.4×20.8 (24.5 - 33.7×19.5 - 22.0) versus 13.5×12.5 (12 - 16×10 - 15) and sporocysts 12.8×8.1 (8.2 - 15.6×6.2 - 12.8) versus 5.6×3.7 (4 - 6×3 - 5) in *E. worleyi*.

Eimeria dunnumi can be recognized as distinct from *E. dauurica*, with oocysts sporting a double layered wall (versus single in *E. dauurica*) and are also significantly larger compared to 20.6×14.1 (17 - 23×13 - 15) for *E. dauurica*. *Eimeria dunnumi* can be recognized as distinct from *E. banfensis* by the smooth oocyst wall and more elongate oocysts (with an average length/width ratio of 1.2) and the lack of a PG. Finally, *E. dunnumi* can be separated from *E. princepsis* by having a double walled oocyst, no PG, and are significantly larger as compared to oocyst size in *E. princepsis* of 21.5×17.3 (19 - 24×15 - 19) (data on *E. princepsis* from HOBBS & SAMUEL (1974).



Figs. 1-6: Digital-images of sporulated oocysts of coccidia recovered from the feces of *Ochotona hyperborea* and *Ochotona pallasii*. *Eimeria burti* n. sp. (1-2). *Eimeria salazarbravoii* n. sp. (3-4). *Eimeria dunnumi* n. sp. (5-6). Scale bar = 10 μ m.

***Eimeria burti* n. sp.**

Diagnosis (Figs. 1, 2, 7):

Oocysts ovoidal ($n = 47$), 26.3×21.1 (SD = 1.1×0.9) ($22.8\text{-}27.89 \times 18.2\text{-}22.6$); length/width ratio of 1.3; wall ($n = 47$) 1.6 (SD = 0.2) ($1.28\text{-}2.07$) of even thickness, outer wall lightly textured, but generally smooth; OR present, but M and PG absent. Sporocysts ($n = 47$) ellipsoidal, tapering 11.4×7.8 (SD = 0.8×0.6) ($8.7\text{-}12.7 \times 6.0\text{-}8.9$); SB present, but SSB, PSB, and SR absent. Sporozoites elongated, side by side; posterior RB present. Age of oocysts at time of study, calculated from time of collection to the date of isolation, was 220 days.

Taxonomic summary

Symbiotype: *Ochotona pallasi* (GRAY, 1867), Museum of Southwestern Biology, MSB 215319, NK 166546 (adult female) (see FREY et al. 1992).

Type locality: Mongolia; Omnogovi Aimag, Gobi Gurvan Saikhan National Park, Tsaagan Ovoo Uul, $43^{\circ} 37.08' \text{ N}$, $103^{\circ} 45.273' \text{ E}$, 2,308 m elevation.

Date collected: 1 July, 2009.

Prevalence: One of 14 (7 %)

Site of infection: Unknown, oocysts recovered from feces.

Material deposited: Phototypes (see BANDONI & DUSZYNSKI 1988) of sporulated oocysts, H.W. Manter Laboratory of Parasitology, HWML49772.

Etymology: This species is named in honor of Dr. M. Scott Burt, a friend, colleague, member of the expedition in 1999, and someone intimately familiar with infections of intestinal parasites.

Remarks

Eimeria burti can be separated from those species that also lack a micropyle by possessing an OR which is absent in *E. worleyi*, *E. pallasi*, *E. daurica*, *E. banfensis*, *E. princepsis*, and *E. dunnumi*. In addition, *E. burti* n. sp. lacks a PG which is present in *E. worleyi*, *E. pallasi*, *E. banfensis*, and *E. princepsis*. *Eimeria burti* n. sp. can be separated from *E. daurica* by the presence of a double layered versus single layered oocyst wall and from *E. dunnumi* by the oocysts being more ovoid in shape and being smaller with oocyst length and width for *E. burti* measuring 26.3×21.1 ($22.8\text{-}27.9 \times 18.2\text{-}22.6$) versus 31.4×20.8 ($24.5\text{-}33.7 \times 19.5\text{-}22.0$) for *E. dunnumi*. These quantitative traits sufficiently distinguish *E. burti* from the other known species of *Eimeria* as to merit its recognition.

***Eimeria salazarbravoi* n. sp.**

Diagnosis (Figs. 3, 4, 8):

Oocysts ovoidal ($n = 17$), 26.6×20.5 (SD = 1.6×1.0) ($24.4\text{-}29.4 \times 18.4\text{-}21.7$); length/width ratio ($n = 17$) 1.3 (SD = 0.1) ($1.2\text{-}1.6$); wall ($n = 17$) 1.6 (SD = 0.2) ($1.38\text{-}1.97$) of even thickness, outer wall generally smooth, but with fine pitting observable at high resolution; M, OR, and PG absent. Sporocysts ($n = 17$) ellipsoidal, tapering 11.6×7.6 (SD = 0.8×0.6) ($10.0\text{-}13.1 \times 6.4\text{-}9.1$); SB and compact SR present, but SSB and PSB absent. Sporozoites elongated and overlapping; posterior RB present. Age of oocysts at time of study, calculated from time of collection to the date of isolation, was 220 days.

Taxonomic summary

Symbiotype: *Ochotona pallasi* (GRAY, 1867), Museum of Southwestern Biology, MSB 215319 NK 166546 (adult female) (see FREY et al. 1992).

Type locality: Mongolia; Omnogovi Aimag, Gobi Gurvan Saikhan National Park, Tsaagan Ovoo Uul, 43° 37.08' N, 103° 45.273' E, 2,308 m elevation.

Date collected: 1 July, 2009.

Prevalence: One of 14 (7 %).

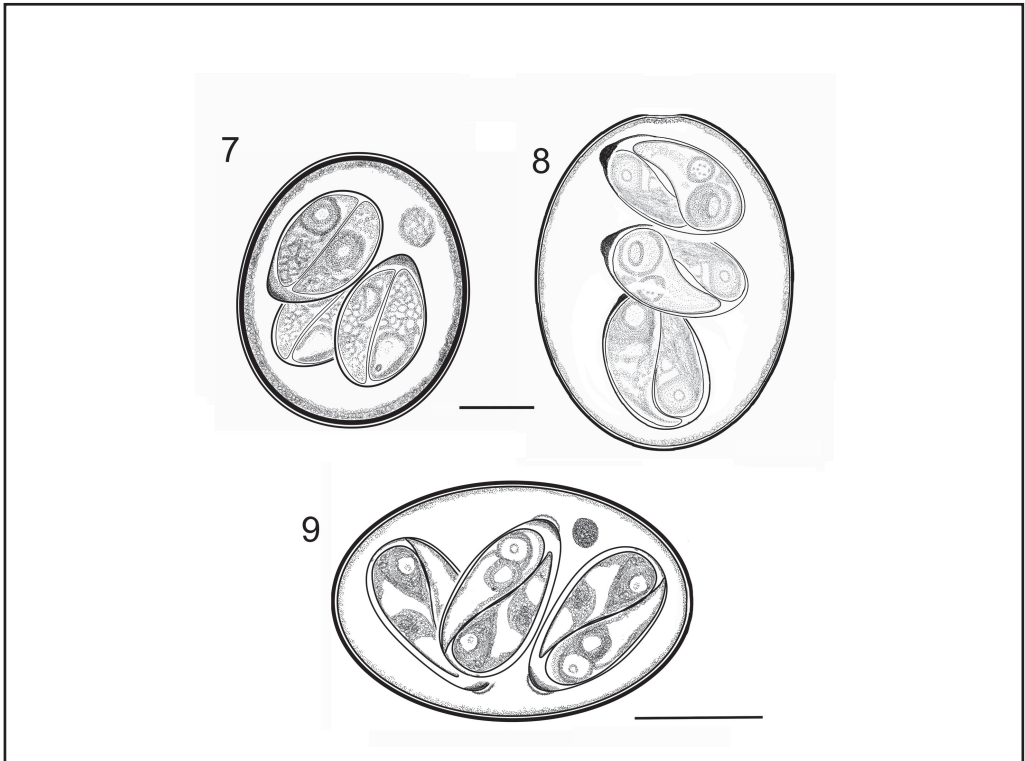
Site of infection: Unknown, oocysts recovered from feces.

Material deposited: Phototypes (see BANDONI & DUSZYNSKI 1988) of sporulated oocysts, H.W. Manter Laboratory of Parasitology, HWML 49773.

Etymology: This species is named in honor of Dr. Jorge Antonio Salazar-Bravo of Texas Tech University, friend, colleague, and member of the 1999 expedition.

Remarks

Eimeria salazarbravo n. sp. superficially resembles *E. haibaiensis*, CAO et al., 2009, recently described from *O. curzoniae*, HODGSON, 1858 from Qinghai Province, China but can be recognized as distinct in the following: oocysts of *E. salazarbravo*, although generally smooth possess



Figs. 7, 8, 9: Line drawings of sporulated oocysts of three species of *Eimeria* recovered from the feces of *Ochotona hyperborea* and *Ochotona pallasii* in Mongolia. Scale bar same for figs. 7 and 8. *Eimeria burti* n. sp. (7), *Eimeria salazarbravo* n. sp. (8), *Eimeria dunnumi* n. sp. (9), scale bar = 10 μ m.

a fine pitting in the external wall which is absent in *E. haibaiensis*. The end of the oocysts is more flattened in *E. salazarbravo* while slightly tapering towards the micropyle in *E. haibaiensis*. The oocysts of individuals of *E. salazarbravo* are also substantially larger, 26.6 x 20.5 (24.4-29.4 x 18.4-21.7) versus 22.2 x 16.2 (20-24 x 15-18).

Eimeria salazarbravo is also somewhat similar to *E. ershovi* MACHULSKII, 1949 described from *O. daurica* in Buryatia. The fine pitting in the oocyst wall is different from the smooth wall described for *E. ershovi*. Oocysts of *E. salazarbravo* are less spherical than *E. ershovi*, length/width ratio of 1.3 (1.2-1.6) versus 1.2 (1.1-1.2), respectively. The oocysts of *E. salazarbravo* are larger in size, 26.6 x 20.5 (24.4-29.4 x 18.4-21.7) versus 21.1 x 18.5 (21-22 x 17-19), as are the sporocysts, 11.6 x 7.6 (10.0-13.1 x 6.4-9.1) versus 9.7 x 5.3 (8-11 x 4-7) for *E. ershovi*. These differences in wall character, shape characteristics, as well as mensural data represent sufficient differences to validate its description as a new species.

A multi-group discriminant analysis on log 10 transformed variables (oocyst length and width, sporocyst length and width, and oocyst wall width) was performed and centroids of all groups were found to be different, with 80.9 % of the variation in the data being accounted for in the first canonical variate (ANONYMOUS 1989). A plot of discriminant scores indicates minimum polygons enclosing the spread of individuals for each species (Fig. 10). The canonical analysis indicates that as the lengths of the oocysts increase, their widths, as well as that of the oocyst wall decrease.

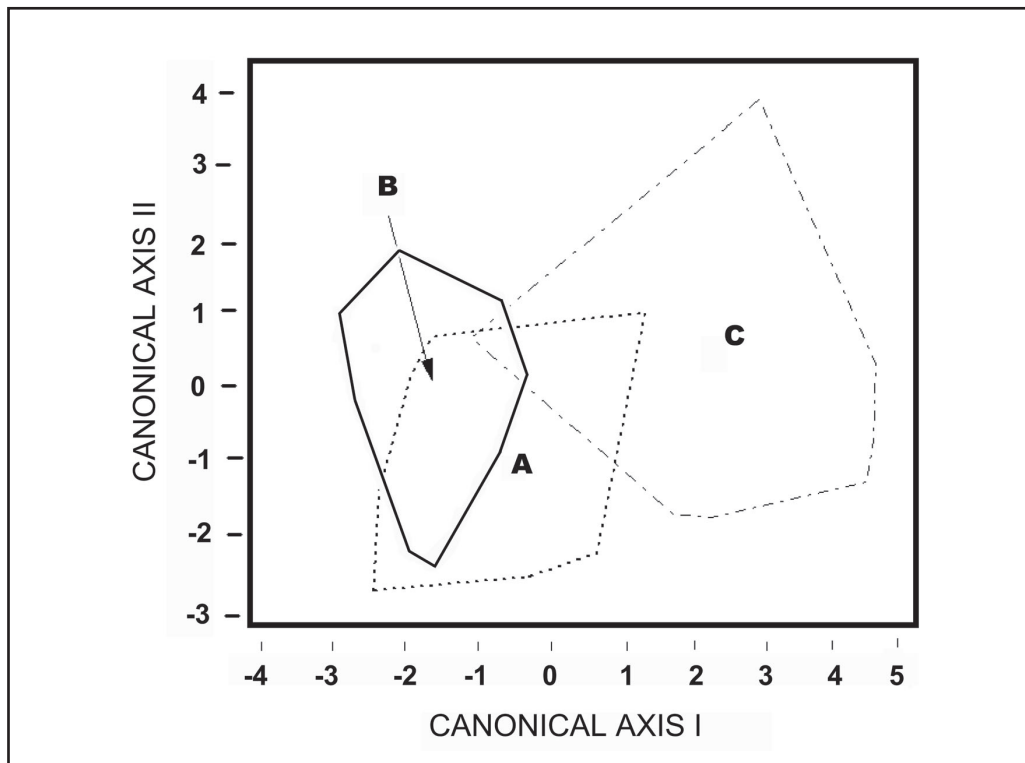


Fig. 10: Plot of discriminant scores of log-10 transformed quantitative-mensural data for the three *Eimeria* spp. described herein. The symbols on the plot represent the multivariate means (centroids) of each of the three new *Eimeria* spp.: A, *E. salazarbravo*; B, *E. burti*; C, *E. dunnumi*.

Discussion

The results of this study add 3 new species of *Eimeria* to the 18 previously described from 7 of the 30 species of *Ochotona* from the Holarctic region (LYNCH et al. 2007, CAO et al. 2009). Seven of the 11 previous studies have focused on 3 species of pika; *O. princeps* and *O. collaris* from localities in Colorado, Canada, and Alaska, and *O. hyperborea* from Japan and northeastern Siberia (LYNCH et al. 2007). The remaining papers have addressed 4 of the 27 remaining species of *Ochotona* from localities scattered across Asia (LYNCH et al. 2007, CAO et al. 2009). LYNCH et al. (2007) noted the similarity of the community of *Eimeria* that infect *O. hyperborea*, *O. princeps*, and *O. collaris*. These 3 species along with *O. pallasi* and *O. alpina* form a clade known as the Northern Group from the studies of YU et al. (2000) and NIU et al. (2004). LYNCH posited that this may reflect a close relationship between the parasites and the hosts in this case, whereas those restricted to the closely related *O. pallasi* may represent a more recent divergence.

The results presented in a paper by CAO et al. (2009) describing the coccidia of *O. curzoniae* from China would tend to disagree with LYNCH et al. (2007). In addition to the description of 2 new species, they found *E. banffensis*, *E. calientinei*, and *E. cryptobaretti*, previously known from the above mentioned Northern Group. Members of *O. curzoniae* belong to the Qinghai-Tibet Plateau Group, a distant clade that also includes *O. dauurica* (YU et al., 2000, 2004). This would seem to indicate that these species of *Eimeria* may not reflect a close evolutionary affinity with their hosts.

As both LYNCH et al. (2007) and CAO et al. (2009) have noted, further investigations are required before conclusions could be drawn from the existing data. Additional work may unveil previously unknown or cryptic species as well as reveal the extent of overlap between hosts. It will be difficult to examine the host/parasite relationships, systematics, and historical ecology of this group until we close the current gap in our knowledge of the communities of *Eimeria* from the species of pikas known from across Asia.

Acknowledgments

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Ochotona pallasi in the region of upper Bulgan-gol (photo: A. STUBBE, 2010).