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Species of Coccidia (Apicomplexa: Eimeriidae) Infecting Pikas from Alaska, U.S.A. and Northeastern Siberia, Russia

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RESEARCH NOTES

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Seroprevalence of *Toxoplasma gondii* Antibodies in Cats From Durango City, Mexico

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ABSTRACT: The prevalence of antibodies to *Toxoplasma gondii* was determined in sera from 105 domestic cats from Durango City, Mexico. Using a modified agglutination test, antibodies to this parasite were found in 21% of the 105 cats, with titers of 1:25 in 3 cats, 1:50 in 4 cats, 1:200 in 5 cats, 1:400 in 2 cats, 1:800 in 2 cats, 1:1,600 in 4 cats, and 1:3,200 or higher in 2 cats. Cats older than 1 yr had a significantly higher frequency of infection than that found in cats younger than 0.5 yr (41 vs. 13.2%, respectively; odds ratio = 4.55; 95% CI = 1.24–17.18; $P = 0.01$). Overall, the seroprevalence of *T. gondii* antibodies in cats in Durango, Mexico, is much lower compared with those reported in other countries.

Cats are essential in the life cycle of *Toxoplasma gondii* because they are the only hosts that can excrete the environmentally resistant oocysts in nature (Dubey and Beattie, 1988). Little is known of the prevalence of *T. gondii* in cats in Mexico. In the present report, we determined the prevalence of *T. gondii* infection in cats from Durango City, Mexico, and we attempted to identify general characteristics of cats associated with infection.

All 105 unwanted cats enrolled from August to November 2006 in the animal shelter in Durango City, Mexico, were studied. The animal shelter receives stray cats captured by the municipality from the streets of Durango City and unwanted pet cats given by the owners for adoption. General data, including age, breed, gender, health status, origin (stray or household), type of food eaten, and residence for cats were obtained (Table I).

The sera were transported by courier from Mexico to Beltsville, Maryland, where serology was performed. Two-fold serial dilutions were made (1:25–1:3,200) and tested with a modified agglutination test (MAT), as described previously (Dubey and Desmonts, 1987). Whole formalin-fixed tachyzoites and mercaptoethanol were used as antigen, and a titer of 1:20 or higher was considered indicative of *T. gondii* exposure based on experimental studies in cats (Dubey and Thulliez, 1989; Dubey et al., 1995a, 1995b).

Results were analyzed with the aid of the software Epi Info 6. For comparison of the frequencies among the groups, the Mantel-Haenszel test, and when indicated the Fisher exact test, were used. The association of the animal characteristics and the *T. gondii* infection was assessed by calculating the odds ratio (OR) with a 95% confidence interval (CI). For age comparison among groups of cats the Student's *t*-test was used. A P value of less than 0.05 was considered significant.

Antibodies to *T. gondii* were found in 21% of the 105 cats, with titers of 1:25 in 3 cats, 1:50 in 4 cats, 1:200 in 5 cats, 1:400 in 2 cats, 1:800 in 2 cats, 1:1,600 in 4 cats, and 1:3,200 or higher in 2 cats.

General characteristics of 105 cats are shown in Table I. All cats were of crossbreed and resided in urban areas. Most cats studied were females, and the frequency of *T. gondii* infection observed in these females was similar to that observed in male cats ($P = 0.56$). Cats were 1 mo to 7 yr old (mean 9 mo), and the prevalence of infection increased with age. Most cats were healthy, and the prevalence of infection in this group did not differ from that in ill cats ($P = 0.33$). In addition, most cats studied were pets. The prevalence of infection in male and female cats in the stray group was comparable (33.3 and 31.3%, respectively; $P = 0.61$). Similarly, although the prevalence of infection in pet female cats (20%) was 2-fold higher than that observed in pet male cats (9.1%), the overall prevalence of infection in male and female cats did not differ significantly ($P = 0.21$).

Cats older than 1 yr had a significantly higher frequency of infection than cats younger than 0.5 yr (41 vs. 13.2%, respectively; OR = 4.55; 95% CI = 1.24–17.18; $P = 0.01$) and slightly higher than that observed in cats 0.5 to 1 yr old (41 vs. 20%; $P = 0.18$). *Toxoplasma gondii* seroprevalence in stray cats is much higher in stray versus pet cats (Dubey, 1973; DeFeo et al., 2002; Nutter et al., 2004) as was the case in the present study. Higher seroprevalence in adult cats versus kittens, observed in the present study, supports earlier findings (Dubey, 1973; Ruiz and Frenkel, 1980b; Pena et al., 2006) and relates to the life cycle of *T. gondii* in cats; most cats are thought to become infected with *T. gondii* after weaning when they begin to hunt for food.

The 21% prevalence of *T. gondii* antibodies in cats of Durango City, Mexico, in the present study is the lowest among all other surveys from North and South America, West Indies, and 1 study from Europe using a cut-off MAT titer of 1:20 (Table II). The prevalence of *T. gondii* in cats is a reflection of prevalence of *T. gondii* in animals that cats access for food. For example, Ruiz and Frenkel (1980a, 1980b) found a very high prevalence of *T. gondii* in cats and rodents and free-range chickens from Costa Rica. Although there are several reports of *T. gondii* infection in humans and animals in Mexico (Varela et al., 1961; Fernandez-Torrano et al., 1986; Velasco-Castrejon et al., 1992; Galvan Ramirez et al., 1995, 1997; Del Rio-Chiriboga et al., 1997; Dubey, Morales, and

TABLE I. General characteristics of the cats and prevalence of *T. gondii* antibodies.

Characteristic	Cats studied		Cats positive	
	No.	%	No.	%
Gender				
Male	34	32.4	6	17.6
Female	71	67.6	16	22.5
Age groups (yr)				
<0.5	53	50.4	7	13.2
0.5–1	30	28.6	6	20
>1	22	21	9	41
Residence area				
Urban	105	100	22	21
Health status				
Healthy	95	90.5	21	22.1
Ill	10	9.5	1	10
Origin				
Stray	28	25.7	9	32.1
Household	77	73.3	13	16.9
Breed				
Crossbreed	105	100	22	21
Food				
Commercial	91	86.7	19	20.9
Homemade	66	62.9	13	19.7
Hunting and garbage	30	28.6	9	30

snails (*Physa* sp.) continued throughout the winter. MacKenzie et al. (1995) cited an abstract of Hoglund and Thulin who reported no change in parasitism.

In summary, exposure of a cold-water, benthic, sedentary flatfish to thermal effluent discharged 5 m above the plume apparently caused no effect on body condition, organ indices, or hematological or pathological alterations. However, prevalence, or mean abundance, or both of some parasites of the fish were affected below the plume, 1 species increasing and 6 species declining. This observation suggests either sensitivity to the effluent or interruption of transmission via their intermediate hosts near the thermal plume. These results provide additional evidence to support the view that some parasites of fish can be useful as bioindicators of subtle environmental changes.

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Species of Coccidia (Apicomplexa: Eimeriidae) Infecting Pikas From Alaska, U.S.A. and Northeastern Siberia, Russia

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ABSTRACT: Eighty-eight fecal samples from 2 species of pika, *Ochotona collaris* and *Ochotona hyperborea,* collected in Alaska (N = 53) and Russia (N = 35), respectively, were examined for the presence of coccidia (Apicomplexa: Eimeriidae). Five oocyst morphotypes were observed. In *O. collaris,* we found *Eimeria calentinei, Eimeria cryptobarretti,* and *Eimeria klondikensis,* whereas in *O. hyperborea,* we found *Eimeria banffensis, E. calentinei, E. cryptobarretti, E. klondikensis,* and *Isospora marquardti.* This study represents new geographic records for all 5 coccidia and new host records for *E. cryptobarretti* and *I. marquardti.* Only minor quantitative differences were seen between the

sporulated oocysts we studied and those reported in their original descriptions.

Pikas are holarctic lagomorphs composed of the single genus, *Ochotona,* with 30 species (Wilson and Reeder, 2005). The majority of species are found in Asia, mainly in the Tibet (Qinghai-Xizang) Plateau region, but also in Afghanistan, Burma, China, India, Iran, Japan, Kazakhstan, Korea, Nepal, Pakistan, and Russia, whereas only 2 species are found in North America (Chapman and Flux, 1990; Yu et al., 2000; Wilson and Reeder, 2005). Currently, 18 coccidia species (16 *Eimeria,* 2 *Isospora*) are described from all *Ochotona* species. Over 3 summer field seasons (2000–2002), the collared pika, *Ochotona collaris* (Nelson, 1893), and the northern pika, *Ochotona hyperborea* (Pallas, 1811), were collected in Alaska and northeastern Siberia, Russia, respectively, as part of the Beringia Coevolution Project (Hoberg et al., 2003; Cook et al., 2005). The present study was conducted to assess the similarity of coccidia fauna in 2 closely related hosts geographically separated by the Bering Strait.

Pikas were caught with museum snap traps or shot with firearms. Fecal specimens were taken from 88 animals from 6 regional field sites: *O. collaris* were collected from 2 sites in Alaska (N = 53), whereas *O. hyperborea* were collected from 4 sites in northeastern Siberia, Russia (N = 35). The Alaskan sites were Wrangell-St. Elias National Park and Yukon-Charley Rivers National Preserve; 4 regions in northeastern Siberia were sampled, the Omolon, Anadyr, and Kolyma river basins and the Providenya Oblast. Symbiotype host specimens (Frey et al., 1992; Brooks, 1993), in which all oocyst species/forms were seen and identified here, are maintained in the University of Alaska Museum of the North (UAM). Feces were preserved in 2.5% (w/v) aqueous $K_2Cr_2O_7$ solution. Oocysts were isolated, measured, and photographed as described by Duszynski and Wilber (1997).

In all, 25% (22/88) of the samples were positive: 12/35 (34%) *O. hyperborea,* and 10/53 (19%) *O. collaris.* Only 4 pikas were host to multispecies infections of coccidia. Five distinct oocyst morphotypes were observed and these were consistent with previously recognized coccidia species from other pikas. Three coccidia species were recovered from *O. collaris*: *Eimeria calentinei, Eimeria cryptobarretti,* and *Eimeria klondikensis*; 5 were recovered from *O. hyperborea*: *Eimeria banffensis, E. calentinei, E. cryptobarretti, E. klondikensis,* and *Isospora marquardti.* The recovery of *E. cryptobarretti* from *O. collaris* and *O. hyperborea* represents 2 new host records. Previously, *E. cryptobarretti* only had been found in *Ochotona princeps,* the American pika, in Colorado (Duszynski and Brunson, 1973). The recovery of *I. marquardti* from *O. hyperborea* also represents a new host record. The recovery of 3 species of coccidia in Alaskan *O. collaris* represents geographic range extensions, as does the recovery of 5 species from *O. hyperborea* in Siberia. Both hosts were studied by Hobbs and Samuel (1974) from pikas collected in the Yukon Territory, Canada, (*O. collaris*) and Japan (*O. hyperborea*); in 92 *O. collaris* they reported *E. banffensis, Eimeria barretti, Eimeria circumborealis, Eimeria princeps, I. marquardti,* and *Isospora yukonensis,* and in 14 *O. hyperborea* they recovered *E. circumborealis, E. princepsis,* and *Eimeria worleyi.*

Because there have been so few published reports of coccidia from these hosts, we include brief mention of qualitative or quantitative (or both) structures as they differ from the original descriptions, along with taxonomic summaries of the species recovered.

Eimeria banffensis Lepp, Todd, and Samuel, 1973

Type host: O. princeps (Richardson, 1828), American pika.

Other hosts (this study): O. hyperborea.

Type locality: North America: Canada: Alberta, Banff, Jumpingpound, and Sibbald creeks.

Geographic distribution: North America: Canada: Alberta, Banff, Jumpingpound, and Sibbald creeks, 51°N, 115°W; Yukon Territory, Ogilvie Mountains, 64°N, 138°W; U.S.A.: Colorado, Larimer and Clear Creek counties; Asia: Japan: Hokkaido, Daisetzusan National Park; Russia: Siberia, Chukotka, 3 km SSE of confluence of Volchya River and Liman Sea, 64°48′N, 177°33′E (this study).

Prevalence: 5/92 (5%) *O. collaris* (type host) in Yukon Territory; 3/14 (21%) *O. hyperborea* in Japan; 5/35 (14%) *O. hyperborea* in Russia (this study); 40/167 (24%) *O. princeps* in Colorado; 11/145 (8%) *O. princeps* in Alberta.

Material deposited: Skull, skeleton, and tissues of a symbiotype host (this study) are preserved in UAM, as UAM no. 84368 (IF 5252), male, 11 August 2002 (collected by N. E. Dokuchaev, A. A. Tsvetkova). Photosyntype of sporulated oocysts are in the U.S. National Parasite Collection (USNPC) as USNPC no. 87390.

Remarks: The morphology of *E. banffensis* from *O. hyperborea* in Russia is similar to the original description provided by Lepp et al. (1973) for this species collected and described from *O. princeps* in Alberta, Canada. Whereas Duszynski and Brunson (1973) described oocysts that were nearly 2 μm smaller in both length and width, the oocyst sizes of our Russian oocysts did not differ when compared with the original specimens (30 × 25 vs. 30 × 25). Duszynski and Brunson (1973) and Hobbs and Samuel (1974) failed to detect the ~2-μm polar granule that was observed in both this study and the original study by Lepp et al. (1973). The recovery of *E. banffensis* is a new geographic record for this parasite in Russia.

Eimeria calentinei Duszynski and Brunson, 1973

Type host: O. princeps (Richardson, 1828), American pika.

Other hosts (this study): O. collaris, O. hyperborea.

Type locality: North America: Colorado, Larimer County.

Geographic distribution: North America: Canada: Yukon Territory, Ogilvie Mountains, 64°N, 138°W, Alberta, 51°N, 115°W; U.S.A.: Colorado: Clear Creek and Larimer counties; Alaska: Yukon-Charley Rivers National Preserve, NW of Rocky Slope of Mt. Kathryn, S of Woodchopper Creek, 65°12′N, 143°33′W (this study); Asia: Japan: Hokkaido, Daisetzusan National Park; Russia: Siberia, Magadanskaya Oblast, 40 km W Magadan, 59°41′N, 150°20′E (this study).

Prevalence: 5/53 (9%) *O. collaris* in Alaska (this study); 8/92 (9%) *O. collaris* in Yukon Territory; 2/35 (6%) *O. hyperborea* in Siberia (this study); 1/14 (7%) *O. hyperborea* in Japan; 2/111 (2%) *O. princeps* in Alberta; 39/167 (23%) *O. princeps* (type host) in Colorado.

Material deposited: Skin, skull, skeleton, and tissues of 2 symbiotype hosts, one for each host species from this study, are preserved in the UAM: *O. collaris,* UAM no. 58399 (AF 49330), male, 1 August 2001 (collected by H. Henttonen, J. Niemimaa, K. Gamblin, L. B. Barrelli) and *O. hyperborea,* UAM no. 80824 (AF 38535), 4 September 2000 (collected by S. O. MacDonald, N. E. Dokuchaev, K. E. Galbreath). Photosyntype of a sporulated oocyst in the USNPC as no. 87393.

Remarks: The morphology of sporulated oocysts of *E. calentinei* from *O. hyperborea* in Russia and *O. collaris* in Alaska is nearly identical to those described by Duszynski and Brunson (1973) for the same species collected from *O. princeps* in Colorado. The recovery of *E. calentinei* establishes new geographic records for this parasite in Russia and Alaska.

Eimeria cryptobarretti Duszynski and Brunson, 1973

Type host: O. princeps (Richardson, 1828), American pika.

Other hosts (this study): O. collaris, O. hyperborea.

Type locality: North America: U.S.A.: Colorado, Larimer and Clear Creek counties.

Geographic distribution: North America: Colorado: Larimer and Clear Creek counties; Alaska: Wrangell-St. Elias National Park (this study), Yukon-Charley Rivers National Preserve, mountainside NW of Headwater Lake of Crescent Creek, 64°82′N, 143°75′W (this study); Asia: Russia: Siberia, Magadanskaya Oblast, mouth of Kegali River, 64°26′N, 161°47′E (this study).

Prevalence: 6/53 (11%) *O. collaris* in Alaska (this study); 5/35 (14%) *O. hyperborea* in Siberia (this study); 107/167 (64%) *O. princeps* (type host) in Colorado.

Material deposited: Skin, skull, skeleton, and tissues of 2 symbiotype hosts, one for each host species from this study, are preserved in the UAM: *O. collaris,* UAM no. 58213 (AF 49535), 18 July 2001 (collected by H. Henttonen, J. Niemimaa, K. Gamblin, L. B. Barrelli) and *O. hyperborea,* UAM no. 80603 (AF 38233), male, 19 August 2000 (collected by S. O. MacDonald, N. E. Dokuchaev, K. E. Galbreath). Photosyntype and photoparatype of sporulated oocysts are in the USNPC, nos. 87480 and 88170, respectively.

Remarks: The morphology of *E. cryptobarretti* from *O. hyperborea* in Russia and *O. collaris* in Alaska is similar to the description by Duszynski and Brunson (1973) for the same species collected from *O.*

TABLE I. Ten studies documenting the presence of coccidia (*Eimeria*, *Isospora* spp.) in pikas (*Ochotona* spp.) from 2 continents.

	North America		Asia			
Coccidia species	*O. collaris*	*O. princeps*	*O. dauurica*	*O. hyperborea*	*O. pallasi*	*O. rufescens*
E. balchanica	—	—	—	—	—	4*
E. banffensis	5	1, 3, 5, 7	—	5, 10	—	—
E. barretti	5	5, 6	—	—	—	—
E. calentinei	5, 10	1, 3, 5	—	5, 10	—	—
E. circumborealis	5	—	—	5	—	—
E. cryptobarretti	10	1, 3	—	10	—	—
E. daurica	—	—	8	—	—	—
E. erschovi	—	—	8	—	9	—
E. klondikensis	5, 10	1, 5	—	5, 10	—	—
E. metelkini	—	—	8	—	—	—
E. ochotona	—	—	8	—	—	—
E. pallasi	—	—	—	—	6	—
E. princeps	5	1, 3, 5	—	5	—	—
E. shubini	—	—	—	—	6	—
E. worleyi	—	1, 6	—	5	—	—
E. sp.	—	—	—	—	6	—
I. marquardti	5	1, 2, 5	—	10	—	—
I. yukonensis	5	—	—	—	—	—

* 1. Duszynski, 1974; 2. Duszynski and Brunson, 1972; 3. Duszynski and Brunson, 1973; 4. Glebezdin, 1978; 5. Hobbs and Samuel, 1974; 6. Lepp et al., 1972; 7. Lepp et al., 1973; 8. Machulsky, 1949; 9. Svanbaev, 1958; 10. Present study.

princeps in Colorado, U.S.A. The recovery of *E. cryptobarretti* establishes new host and geographic records for this parasite in Russia and Alaska, U.S.A. The authors of the original description hesitated to state if there was a micropyle on the oocyst, but we now believe that *E. cryptobarretti* does indeed have one.

Eimeria klondikensis Hobbs and Samuel, 1974

Type host: O. collaris (Nelson, 1893), collared pika.

Other hosts (this study): O. hyperborea.

Type locality: North America: Canada: Yukon Territory, Ogilvie Mountains, 64°N, 138°W.

Geographic distribution: North America: Canada: Yukon Territory, Ogilvie Mountains, 64°N, 138°W; Alberta, 51°N, 115°W; U.S.A.: Colorado: Clear Creek County; Alaska: Wrangell-St. Elias National Park and Preserve, SE of Rock Lake, 21 July 2001, 61°47′N, 141°12′W (this study); Yukon-Charley Rivers National Preserve (this study); Asia: Japan: Hokkaido, Daisetzusan National Park; Russia: Siberia, Chukotka, 3 km SSE of confluence of Volchya River and Liman Sea, 64°48′N, 177°33′E (this study).

Prevalence: 2/53 (4%) *O. collaris* in Alaska (this study); 3/92 (3%) *O. collaris* (type host) in Yukon Territory; 1/35 (3%) *O. hyperborea* in Siberia (this study); 2/14 (14%) *O. hyperborea* in Japan; 7/111 (6%) *O. princeps* in Alberta; 62/224 (28%) *O. princeps* in Colorado.

Material deposited: Skin, skull, skeleton, and tissues of 2 symbiotype hosts, one for each host species from this study, are preserved in the UAM: *O. collaris,* UAM no. 56067 (AF 54551), female, 21 July 2001 (collected by S. Kutz, A. Tsvetkova, A. A. Eddingaas, M. McCain) and *O. hyperborea,* UAM no. 84369 (IF 5253), male, 11 August 2002 (collected by N. E. Dokuchaev, A. A. Tsvetkova). We deposited a photoneotype of a sporulated oocyst in the USNPC as no. 99671, because no previous authors had archived a type specimen of this parasite.

Remarks: The morphology of *E. klondikensis* from *O. collaris* in Alaska and *O. hyperborea* in Russia is similar to the description provided by Hobbs and Samuel (1974) for the same species collected and described from the same hosts in Canada and Japan, respectively. The recovery of *E. klondikensis* establishes new geographic records for this parasite in Russia and Alaska, U.S.A. Both a line drawing and a photomicrograph of the sporulated oocyst of this species appeared in the original description.

Isospora marquardti Duszynski and Brunson, 1972

Type host: O. princeps (Richardson, 1828), American pika.

Other hosts (this study): O. hyperborea.

Type locality: North America: U.S.A.: Colorado, Ft. Collins, Clear Creek, and Larimer counties.

Geographic distribution: North America: U.S.A.: Colorado, Ft. Collins, Clear Creek, and Larimer counties; Canada: Yukon Territory, Ogilvie Mountains, 64°N, 138°W; Alberta, 51°N, 115°W; Asia: Russia: Siberia, Chukotka, Ulhum River, 15 km W of Chaplino Village, 64°25′N, 172°32′E (this study).

Prevalence: 1/92 (1%) *O. collaris* in the Yukon Territory (this study); 1/35 (3%) *O. hyperborea* in Siberia (this study); 1/111 (<1%) *O. princeps* in Alberta; 25/167 (15%) *O. princeps* (type host) in Colorado.

Material deposited: Skull, skeleton, and tissues of a symbiotype host from this study are preserved in the UAM as UAM no. 83836 (IF 7569), female, 28 July 2002 (collected by V. F. Fedorov, K. E. Galbreath). Photosyntype of a sporulated oocyst is in the USNPC as no. 87408.

Remarks: The morphology of sporulated oocysts of *I. marquardti* from *O. hyperborea* in Russia differ slightly from those of Duszynski and Brunson (1972) collected and described from *O. princeps* in Colorado; the latter had oocysts and sporocysts that were larger in both length and width (31 × 30 and 19 × 12 vs. 28 × 27 and 17 × 11) than those of our Russian specimens. Still, both oocysts and sporocysts reported here were larger than those measured by Hobbs and Samuel (1974) from *O. collaris* (23 × 22 and 15 × 9). Oocysts of some species are known to exhibit phenotypic plasticity (see Duszynski et al., 1992) and, given the similarity of qualitative data, we believe these oocysts are *I. marquardti.* The recovery of *I. marquardti* establishes a new host and geographic record for this parasite in Russia.

Machulsky (1949) published the first paper on coccidia in pikas. Since then, 9 additional papers, including this one, have described a total of 18 coccidia species in 6 *Ochotona* species: 2 from North American and 4 from Asia (Table I). The coccidia reported from 3 of those 6 hosts, *O. collaris, O. princeps,* and *O. hyperborea,* which are the best studied hosts (Table I), are remarkably similar. These hosts have all been studied on multiple occasions and 10 coccidia have been reported from them. Six of 10 (60%) coccidia have been reported from all 3 hosts, whereas 3 others have been reported from at least 2 hosts. One species, *Isospora yukonensis,* has been reported from only a single individual of *O. collaris* (Hobbs and Samuel, 1974). The overlap of coc-

cidia species among *O. collaris, O. hyperborea,* and *O. princeps* suggests the possibility that these coccidia may have evolved from a common ancestor, i.e., that shared coccidia faunas in 3 closely related pika species may reflect a single origin for the parasites in their common ancestor. On the other hand, this pattern may indicate that each coccidium had a common ancestor in the ancestor of the pikas. Thus, the parasite community may have a recent origin, but this doesn't say anything about relationships among these coccidia.

Except for *Eimeria erschovi* Machulsky, 1949, the 7 coccidia identified from the remaining Asian pikas, *Ochotona dauurica* (*Eimeria daurica, Eimeria metelkini, Eimeria ochotona,* in 1949), *Ochotona pallasi* (*Eimeria pallasi, Eimeria shubini, Eimeria* sp., in 1958), and *Ochotona rufescens* (*Eimeria balchanica,* in 1978), only have been identified once, each from 2–3 specimens of their single host species. Initially, the lack of overlap indicates that these coccidia may be more host-species specific, but nothing substantive is known about host specificity in pika coccidia. On the other hand, given the known distributions of the 3 host species, it is unlikely that these coccidia would ever come into contact with an *Ochotona* species different from the one in which it was first described; *O. rufescens,* the Afghan pika, is geographically separated from the other 2 species and, although the ranges of *O. dauurica* and *O. pallasi* share some overlap, e.g., Mongolia, these species are separated both by altitude and biome (high mountain vs. desert, respectively). In addition, the obvious sampling bias doesn't allow meaningful comparisons. Finally, the question must be asked whether any of these 7 coccidia even still exist since 2 of the 3 host species are either endangered (*O. pallasi*) or threatened (*O. rufescens*).

Our a posteriori hypothesis was that the similarity, or disparity, of coccidia infecting pikas would reflect the systematics and phylogenetics of the hosts. Work by Yu et al. (2000) on the phylogeny of 19 pika species included *O. hyperborea, O. princeps, O. pallasi,* and *O. dauurica*; sequences from *O. collaris* and *O. rufescens* were not incorporated. The data of Yu et al. (2000) indicated that there are 3 pika clades: a shrub-steppe group of 7 species (including *O. dauurica*), a northern group of 5 species (including *O. hyperborea, O. pallasi,* and *O. princeps*), and a mountain group of 7 species. Host–parasite data to date (Table I) support the notion that *O. hyperborea* and *O. princeps* may be infected by the same coccidia because they have descended from a recent common ancestor. If true, this would predict that the same or similar coccidia species will be found in other species from the northern group of Yu et al. (2000). In other words, the morphological similarity of the coccidia in this study might reflect close phylogenetic relationships that are a consequence of the close relationship between the hosts.

The data of Yu et al. (2000) posit that *O. princeps* is the most basal member of the northern group. Interestingly, *O. pallasi* is infected by an entirely different set of coccidia from other hosts in the northern clade. In fact, *O. pallasi* is infected by *E. erschovi,* a coccidium first identified from *O. dauurica,* a member of the shrub-steppe group of pikas. Despite an older association between *O. dauurica* and *O. pallasi,* it is possible that *E. erschovi* is a generalist parasite capable of a broad co-accommodation of hosts (Brooks, 1979). In other words, the association between *E. erschovi* and 2 deeply divergent pika lineages may suggest the generalist nature of this coccidium. These hosts are in relatively close contact as the range of *O. pallasi* overlaps that of *O. dauurica,* although they occupy different habitats (Chapman and Flux, 1990); unfortunately, it is not known if any burrowing or talus-dwelling pikas live in enough proximity to connect these pika lineages. Perhaps the other *Eimeria* spp. identified from *O. pallasi* (*Eimeria pallasi, Eimeria shubini,* and *Eimeria* sp.) are more derived (than those infecting *O. princeps*) and are results of recent speciation. This would keep the cospeciation hypothesis alive, but it is also possible that a host switch could have led to this association. Only phylogenetic (sequence) data for these coccidia will resolve the relationships among them.

It also is recognized that the dichotomy seen in Table I, where 3 hosts overlap in eimeriid fauna and the other 3 hosts have divergent fauna, could be the result of poor species descriptions. Both Hobbs and Samuel (1974) and Lepp et al. (1972) addressed this possibility. Hobbs and Samuel (1974) noted the extreme similarity between many of the ''continental Asian'' and the ''North American'' coccidia. In all cases, there were enough differences among those coccidia to prevent the synonymy of species. Before conclusions can be made regarding the validity of species descriptions, more Asian pikas must be surveyed. Finding evidence for cryptic species of coccidia in pikas also could be an important issue for sorting out the origins of their host–parasite associations.

In conclusion, we emphasize 3 major points: (1) the similarity in coccidia fauna among *O. princeps, O. collaris,* and *O. hyperborea*; (2) the different and more diverse coccidia parasites in Asian hosts; and (3) the apparent widespread species of coccidia found in pikas representing 2 different host clades.

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A *Ribeiroia* Spp. (Class: Trematoda)—Specific PCR-Based Diagnostic

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ABSTRACT: Increased reporting of amphibian malformations in North America has been noted with concern in light of reports that amphibian numbers and species are declining worldwide. *Ribeiroia ondatrae* has been shown to cause a variety of types of malformations in amphibians. However, little is known about the prevalence of *R. ondatrae* in North America. To aid in conducting field studies of *Ribeiroia* spp., we have developed a polymerase chain reaction (PCR)-based diagnostic. Herein, we describe the development of an accurate, rapid, simple, and cost-effective diagnostic for detection of *Ribeiroia* spp. infection in snails (*Planorbella trivolvis*). Candidate oligonucleotide primers for PCR were designed via DNA sequence analyses of multiple ribosomal internal transcribed spacer-2 regions from *Ribeiroia* spp. and *Echinostoma* spp. Comparison of consensus sequences determined from both genera identified areas of sequence potentially unique to *Ribeiroia* spp. The PCR reliably produced a diagnostic 290-base pair (bp) product in the presence of a wide concentration range of snail or frog DNA. Sensitivity was examined with DNA extracted from single *R. ondatrae* cercaria. The single-tube PCR could routinely detect less than 1 cercariae equivalent, because DNA isolated from a single cercaria could be diluted at least 1:50 and still yield a positive result via gel electrophoresis. An even more sensitive nested PCR also was developed that routinely detected 100 fg of the 290-bp fragment. The assay did not detect furcocercous cercariae of certain Schistosomatidae, *Echinostoma* sp., or *Sphaeridiotrema globulus* nor adults of *Clinostomum* sp. or *Cyathocotyle bushiensis*. Field testing of 137 *P. trivolvis* identified 3 positives with no overt environmental cross-reactivity, and results concurred with microscopic examinations in all cases.

Concern over declining numbers and species of amphibians has come to the forefront over the past 20 yr (Barinaga, 1990; Blaustein and Wake, 1990; Phillips, 1990; Pechmann et al., 1991; Wake, 1998). Suggested factors, singly or in synergism, that have been hypothesized as reasons for the decline of this class of animals include habitat destruction (Kolozsvary and Swihart, 1999; Houlahan and Findlay, 2003), UV irradiation (Blaustein et al., 1998, 2003), introduced species (Knapp and Mathews, 2000), climate change (Beebee, 1995; Corn, 2005), and various pathogens (Daszak et al., 2003). A current review of the factors is found in Beebee and Griffiths (2005). Amphibian malformations are of growing concern, because they have been observed with increased prevalence in North America (Ouellet, 2000). Although malformations have the potential to deleteriously affect populations or species at particular sites, they have not been empirically linked to global or regional declines. Recent reports (Johnson et al., 1999, 2002; Lannoo et al., 2003; Schoff et al., 2003; Schotthoefer et al., 2003) have implicated the trematode *Ribeiroia ondatrae* as a causative agent of some types of malformations. Little is known about the distribution of this parasite in its hosts within North America. Wilson et al. (2005) identified 3 species of *Ribeiroia*: *R. ondatrae* within the Americas; *R. marini* in the Carribbean, and *Cercaria lilet*a in Africa. *Ribeiroia ondatrae* has a 3-host life cycle with 2 aquatic intermediate hosts and a predator definitive host, usually a bird or mammal. *Planorbella* spp. serves as first intermediate host, with fish and various amphibians as second intermediate hosts. Exogenous factors, which include pesticides (Kiesecker, 2002), and eutrophication, which leads to a dominance of *Planorbella* spp. (Johnson and Chase, 2004), have been shown to increase malformation rates. Currently, *Ribeiroia* spp. infections in the first intermediate host are diagnosed by dissection of live or freshly dead snail hosts for various larval stages, which requires training and substantial time to locate infected tissues to identify the parasite correctly. Identifying larvae early in development after miracidial penetration but before the development of the cercariae is difficult, if not impossible, using morphological characters. To increase the speed and accuracy in the examination of large numbers of snails for the presence of *Ribeiroia* spp., to reduce labor costs, and to simplify training required, we have developed a genus-specific polymerase chain reaction (PCR)-based diagnostic that targets the second internal transcribed spacer (ITS-2) region of the ribosomal RNA gene cluster (Morgan and Blair, 1998; Kostadinova et al., 2003; Wilson et al., 2005). By using various combinations of 4 oligonucleo-

TABLE I. Oligonucleotides and PCR profiles used to detect *Ribeiroia* sp.

Primer	Sequence	T_m	% GC	Use/product size
21-up	AGTCATGGTGAGGTGCAGTGA	59.7	52.4	with 18-dn, 290 bp, profile 1
18-dn	AGACCGCTTAGATAGCAG	51.4	50.0	with 21-up, 290 bp, profile 1
18-up	CGTGTTTGGCGATTTAGT	51.4	44.4	Nested reaction with 19-dn, 164 bp, profile 2
19-dn	TCAAAAATGAAGCAACAGT	49.1	31.6	Nested reaction with 18-up, 164 bp, profile 2

Profile 1								
1X	94 C	4 min						
	94 C	15 sec		94 C	15 sec			
10X	59 C	30 sec	26X	59 C	30 sec	1X	72 C	7 min
	72 C	45 sec		72 C	90 sec		4 C	Hold

Profile 2. As above except anneal at 53 C versus 59 C.