1999

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ELSEVIER

International Journal for Parasitology 29 (1999) 275–284

INTERNATIONAL Journal for PARASITOLOGY

Three new species of *Eimeria* from Bolivian marsupials

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Received 13 July 1998; received in revised form 6 October 1998; accepted 7 October 1998

Abstract

Faecal samples collected from 300 Bolivian marsupials (Didelphimorphia: Didelphidae) between 1984 and 1993 were examined for coccidian parasites. Sporulated oocysts were present in the faeces of 50 (17%) marsupials representing 11 genera and 22 species. Three new species of *Eimeria* are described and named from six host species. One species occurred in *Marmosops dorothea*, *Monodelphis domestica* and *Thylamys venustus*, another in *Micoureus constantiae constantiae* and *Micoureus constantiae budini* and a third in *Marmosops dorothea*. A discriminant analysis performed on five quantitative oocyst measurements revealed similarities between the first and third *Eimeria* species because of similar sizes and shapes of the oocysts, whereas the second *Eimeria* species was structurally discrete. The *Eimeria* that infects multiple hosts may be a common widespread species. Future surveys are advised for a thorough assessment of the coccidian biodiversity within Bolivian marsupials. © 1999 Australian Society for Parasitology. Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Eimeria*; Apicomplexa; Marsupial; Didelphidae; *Thylamys*; *Marmosops*; *Monodelphis*; *Micoureus*; New species; Bolivia

1. Introduction

The extant marsupial species in South America represent the remnants of an extensive adaptive diversification that occurred after their separation from placental mammals more than 100 Myr ago [1]. The oldest fossil marsupials are from the late Cretaceous of North America [2, 3], indicating a northern origin and subsequent southward dispersal into South America, Antarctica, and finally into Australia in about the early Oligocene [1, 4, 5]. The presence of *Didelphis virginiana* Kerr in North America, the only didelphid occurring north of Mexico, is a result of a relatively recent dispersal event that occurred after the closing of the isthmus of Panama [6].

Members of the family Didelphidae are considered to be the most primitive of the extant taxa of marsupials [1]. Many workers consider the Didelphidae to represent the original group from which all other marsupials are derived [1, 7]. Literature on the coccidian parasites report that only six *Eimeria* species have been recorded from didelphids in Brazil and the USA [8–12].

Herein we describe three new *Eimeria* species, based on the structure of their sporulated oocysts, found in opossum hosts of the family Didelphidae from localities throughout Bolivia, South America.

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0020-7519/99/$ - see front matter
PII: S0020-7519(98)00199-4

2. Materials and methods

Fresh faecal samples were collected from live-trapped marsupials captured at over 40 localities in Bolivia during the years 1984–1993 and transported to The University of New Mexico (UNM). Voucher specimens in the form of skins, skeletons and tissues are permanently deposited in the Museum of Southwestern Biology (MSB, UNM), Albuquerque, NM or the Collection Boliviana de Fauna (CFB), La Paz, Bolivia. Symbiotype hosts, those specimens from which new eimerian species were first isolated, are curated in a separate section in the MSB [13].

Faecal samples were placed in separate vials containing 2% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) and processed following Duszynski and Wilber [14]. Sporulated oocysts were 92–3992-days-old when measured. The number of layers in the oocyst walls was determined for each species by using friction of the coverglass to fracture and separate the layers of the oocyst wall. Photosyntypes [15] of sporulated oocysts were deposited in the United States National Parasite Collection (USNPC), Beltsville, MD. A multigroup discriminant analysis was performed using Statistical Analysis Systems (SAS version 6.07) on five quantitative measurements of the three *Eimeria* taxa described in this study [16]. All measurements are in μm, with size ranges in parentheses following the means.

3. Results

Faeces from 300 marsupials belonging to 22 species in 11 genera were collected at 40 localities in Bolivia and examined for coccidian oocysts. Oocysts were present in 50 of 300 (17%) individuals. No coccidia were found in 16 host species ($n=104$) including: *Caluromys lanatus lanatus* (1), *Chironectes minimus minimus* (2), *Didelphis albiventris albiventris* (6), *D. a. pernigra* (5), *Didelphis marsupialis* (11), *Gracilianus agilis buenavistae* (8), *G. a. chacoensis* (1), *Marmosa murina* (1), *Marmosops impavidus* (3), *Marmosops noctivagus keaysi* (8), *Marmosops parvidens* (2), *Metachirus nudicaudatus bolivianus* (3), *Monodelphis brevicaudata* (3), *Monodelphis kunsi* (4), *Philander opossum canus* (28) and *Thylamys pusillus* (18). However, 50 of 196 (26%) hosts, representing six other host species, collected at 20 localities in seven of Bolivia's nine departments, had oocysts in their faeces (Fig. 1, Table 1); these represent three new *Eimeria* species which are described below. One eimerian species was found in three host species from three genera, another in two host species from one genus, while the other two species were found only in a single host species (Tables 1–2). A double infection occurred only once, in *Thylamys venustus* Thomas, which contained two of the morphotypes we describe (Table 1). Unsporulated eimerian oocysts were detected in another 12 animals, but could not be identified to species level (Table 1).

3.1. Eimeria cochabambensis n. sp. (Figs 2–4, 11)

3.1.1. Species description

Sporulated oocyst subspheroidal, wall ~2.0 (1.2–2.5) thick, composed of two layers: outer sculptured, yellow, appears slightly striated in cross section, ~3/4 of total thickness; inner transparent, green or pink; micropyle and oocyst residuum absent; large, refractile polar granule present; sporulated oocysts ($n=150$) 21.6×20.2 (17–27×17–24) with length/width ratio (L/W) 1.1 (1.0–1.2); sporocysts fusiform, 11.0×7.2 (8–13×4–8) with L/W 1.5 (1.2–2.0); prominent Stieda body present, but not sub- or parastieda bodies; sporozoites lie at each end of sporocyst with residuum consisting of a slightly flattened globular mass between them; one large refractile body at either end of sporozoites.

3.1.2. Diagnosis

Eimeria cochabambensis was present in three host species each in a separate genus (Tables 1–2); *Eimeria* species previously have not been reported from these host genera. Even though there are slight size differences in the oocysts collected from hosts in the different genera (Table 2), the same morphological characteristics are found in all of the oocysts. Only six *Eimeria* species have been described from hosts in Didelphidae [8–12]. Sporulated oocysts of *E. cochabambensis* are slightly smaller in size than those of *Eimeria philanderi* Lainson and Shaw from *Philander opossum opossum* Linnaeus (21.6×20.2

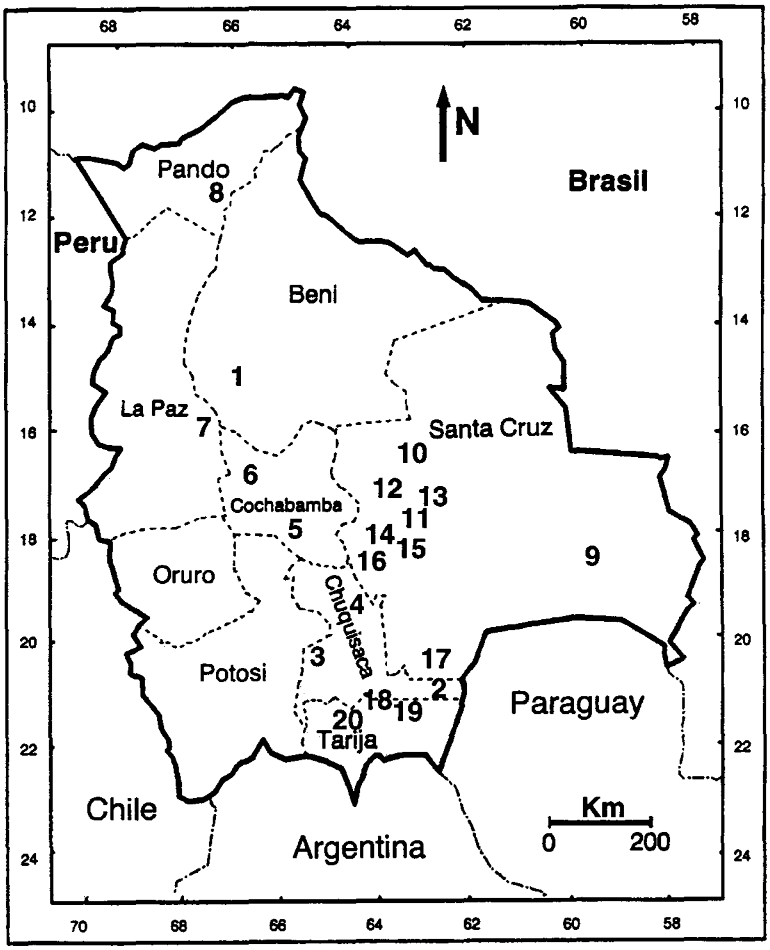

Fig. 1. Map of Bolivia showing its nine departments and surrounding countries within South America. The numbers indicate the approximate location of captured marsupial hosts, in which *Eimeria* spp. were found, corresponding to collection locality Nos in Table 1.

vs 23.5 × 22.4, respectively), as are its sporocysts. They both have a two-layered wall, a polar granule and both lack a micropyle and an oocyst residuum. However, the wall of *E. philanderi* is described as having a mamillated outermost surface with two striated layers, the outer colourless and the inner one brown-yellow [12]. The wall of *E. cochabambensis* also has two layers with the outer layer sculptured and slightly striated, but the inner layer is transparent. The sporocysts of this species have a distinct, large sporocyst residuum and the sporozoites have large refractile bodies, while those of *E. philanderi* have a sporocyst residuum of numerous, small granules and no refractile bodies in the sporozoites. *Eimeria cochabambensis* has a large and highly refractile polar granule lacking in *E. philanderi*. This species was the most common coccidal infection encountered, as it was found in samples from 28 hosts in four departments and was collected in three of the 10 sampling years (Table 1).

3.1.3. Type materials

Symbiotype: *Marmosops dorothea* Thomas, 1911, mouse opossum.

Type locality: SOUTH AMERICA, Bolivia, Cochabamba, 9.5 km by road NE of Tablas Monte, Rio Jatun Mayu 17°02′29″S, 65°59′05″ W, elevation (elev.) 1500 m (No. 6 in Fig. 1), 15 July 1993.

Table 1
Collection localities and dates of marsupial hosts taken in Bolivia, South America, from 1984 to 1993 and the *Eimeria* species found in each host species

Department and collection localities[a]		Host species	No. infected/ No. examined (%)	*Eimeria* species (No. of hosts)[b]	Date collected
Beni	1	*Marmosops dorothea*	0/1		92
Chuquisaca	2	*Monodelphis domestica*	7/19 (37)	*E. cochabambensis*	85
		Thylamys pallidior	0/1		85
		Thylamys venustus	9/28 (32)	*E. cochabambensis* (8), *E.* sp. (2)[c,d]	85
	3	*T. pallidior*	1/2 (50)	*E.* sp.	86
	4	*Micoureus constantiae budini*	0/1		90
		M. domestica	0/2		90
		T. venustus	2/13 (15)	Unsporulated oocysts	90
Cochabamba	5	*T. venustus*	0/3		91
	6	*M. dorothea*	8/18 (44)	*E. cochabambensis* (7), Unsporulated oocysts (1)	93
		Micoureus constantiae constantiae	4/6 (67)	*E. micouri*	93
		T. pallidior	0/2		93
La Paz	7	*M. c. budini*	0/8		92
		M. dorothea	0/2		92
Pando	8	*M. c. constantiae*	0/1		86
Santa Cruz	9	*M. domestica*	0/1		84
	10	*M. dorothea*	2/5 (40)	*E. cochabambensis* (1), *E. marmosopos* (1)	85
	11	*M. dorothea*	1/4 (25)	*E. marmosopos*	87
	12	*M. dorothea*	1/2	Unsporulated oocysts	87
	13	*M. dorothea*	0/3		88
		M. c. budini	0/1		88
	14	*M. dorothea*	0/8		84, 91
		M. c. budini	1/1 (100)	*E. micouri*	91
	15	*T. venustus*	3/18 (17)	*E. cochabambensis* (2), Unsporulated oocysts (1)	91
	16	*T. venustus*	0/2		90
	17	*M. domestica*	0/4		91
Tarija	18	*T. venustus*	0/7		86, 91
	19	*T. venustus*	3/5 (60)	*E. cochabambensis* (1), Unsporulated oocysts (2)	91
		M. c. budini	1/1 (100)	*E. micouri*	91
	20	*T. venustus*	7/27 (26)	*E. cochabambensis* (2), Unsporulated oocysts (5)	91
20 Localities		6 Host spp.	50/196 (26)	4 *Eimeria* spp.	

[a] Numbers correspond to Fig. 1.
[b] Numbers in parentheses after the *Eimeria* species indicate hosts infected with each *Eimeria* species.
[c] One individual had a double infection.
[d] *E.* sp. represents a fourth *Eimeria* species, which had too few oocysts to describe adequately.

Table 2
Mensural characters of sporulated oocysts of *Eimeria cochabambensis* from three host species[a]

	Oocyst				Sporocyst			Wall
Host spp.	*n*	Length	Width	L/W	Length	Width	L/W	Thickness
Marmosops dorothea[b]	50	21.1 (17–24)	19.8 (17–22)	1.1 (1–1.2)	11.0 (9.5–12.5)	7.7 (6.5–9)	1.4 (1–1.6)	1.8 (2–2.5)
Monodelphis domestica	50	20.4 (17.5–24)	19.6 (17–24)	1 (1–1.1)	10.5 (8–11.5)	6.5 (4–7)	1.6 (1.5–2)	1.5 (1–2)
Thylamys venustus	50	23.3 (19–26.5)	21.4 (18–22.5)	1.1 (1–1.2)	11.6 (10–13)	7.3 (6.5–8)	1.6 (1.3–1.7)	2.3 (1.8–2.5)
Combined	150	21.6 (17–26.5)	20.2 (17–24)	1.1 (1–1.2)	11.0 (8–13)	7.2 (4–8)	1.5 (1.2–2)	2.0 (1.2–2.5)

[a] All measurements in μm, with size ranges in parentheses below the means.
[b] Denotes type host.

Material deposited: Photosyntype [15] of sporulated oocysts in the USNPC No. 88157. Symbiotype [13]: *Marmosops dorothea* in the MSB, UNM No. 87080 (NK 30323, female), M.L. Campbell No. 2461, 15 July 1993.

3.1.4. Site of infection

Unknown. Oocysts collected from faeces.

3.1.5. Other hosts and localities

Monodelphis domestica Wagner, 1842, short tailed opossum; *T. venustus* Thomas, 1902, mouse opossum. See Table 1 and Fig. 1 for localities.

3.1.6. Prevalence

See Table 1.

3.1.7. Etymology

The nomen triviale is derived from the [Departamento de] Cochabamba, where the first infected host was collected, and *-ensis* (L., belonging to).

3.2. *Eimeria micouri* n. sp. (Figs 5–7 and 12)

3.2.1. Species description

Sporulated oocyst ellipsoidal, wall ~1.6 (1.2–2.0) thick, composed of two equally thick layers: outer pitted; inner transparent; micropyle and oocyst residuum absent; one or two polar granules present; sporulated oocysts ($n = 50$) 24.6 × 18.2 (20–28 × 17–20) with L/W 1.3 (1.2–1.5); sporocysts fusiform, 11.5 × 6.7 (10–13 × 6–8) with L/W 1.7 (1.5–1.8); prominent Stieda body present, but not sub- or parastieda bodies; sporocyst residuum of several small globules usually along one margin of sporocyst; sporozoites each with one large, posterior refractile body and one small, anterior refractile body and lie lengthwise in sporocyst, head-to-tail.

3.2.2. Diagnosis

No *Eimeria* species have been reported from this host genus. *Eimeria micouri* resembles *Eimeria haberfeldi* Carini, described from *Caluromys philander* Linnaeus (Subfamily Caluromyinae) because of its ellipsoidal shape, absence of an oocyst residuum and presence of a Stieda body. However, it differs from *E. haberfeldi* by being smaller (25 × 18 vs 30 × 20), by having a two-layered wall vs a one-layered wall, and by having polar granules which *E. haberfeldi* lacks [9]. This species was present in the two *Micoureus constantiae* subspecies found in three departments, during the 2 years sampled (Table 1).

3.2.3. Type materials

Symbiotype: *Micoureus constantiae constantiae* Thomas, 1904, mouse opossum.

Type locality: SOUTH AMERICA, Bolivia, Cochabamba, 9.5 km by the road NE of Tablas Monte, Rio Jatun Mayu 17°02′29″S, 65°59′05″W, elev. 1500 m (No. 6 in Fig. 1).

Material deposited: Photosyntype of sporulated oocysts in USNPC, No. 88159. Symbiotype *Micoureus constantiae constantiae* in the CFB, No. 3569 (NK 30341, male), J.P. Téllez No. 25, 16 July 1993.

3.2.4. Site of infection
Unknown. Oocysts collected from faeces.

3.2.5. Other hosts and localities
Micoureus constantiae budini Thomas 1919, mouse opossum. See Table 1 and Fig. 1 for localities.

3.2.6. Prevalence
See Table 1.

3.2.7. Etymology
The nomen triviale is derived from the generic part of the scientific name of the host, in the genitive singular ending, meaning "of *Micoureus*".

3.3. Eimeria marmosopos n. sp. (Figs 8–10 and 13)

3.3.1. Species description
Sporulated oocyst subspheroidal, wall ~2.2 (1.8–2.5) thick, composed of one layer: rough, striated; micropyle and oocyst residuum absent; highly refractile polar granule present; sporulated oocysts ($n=52$) 22.2 × 19.9 (19–25 × 17–23) with L/W 1.1 (1.0–1.2); sporocysts ovoidal, 11.1 × 6.8 (8–13 × 5–8) with L/W 1.7 (1.3–2.0); Stieda body and substieda body present, but not parastieda body; sporocyst residuum present, consisting of several large globules in centre or to one side of sporocyst; sporozoites lie head-to-tail with one posterior refractile body each.

3.3.2. Diagnosis
No *Eimeria* species have been recorded from this host genus. Sporulated oocysts of *Eimeria marmosopos* are similar in oocyst shape (subspheroidal) and size to those of *E. cochabambensis* and *E. philanderi* (22 × 20 vs 22 × 20 vs 24 × 22, respectively). *Eimeria philanderi* and *E. cochabambensis* have two-layered walls, a polar granule, and their sporocysts have Stieda bodies. The wall of *E. marmosopos* appears two-layered, although when it is fractured (see Methods) we are able to visualise only one layer. *Eimeria marmosopos* has a thick striated single-layered wall, a highly refractile polar granule, and a substieda body, which the other two species lack; it was found only in one host species from one department in 1985 and 1987 (Table 1).

3.3.3. Type materials
Symbiotype: *Marmosops dorothea* Thomas, 1911, mouse opossum.
Type locality: SOUTH AMERICA, Bolivia, Santa Cruz, 15 km S of Santa Cruz 17°53′S, 63°07′W, elev. 400 m (No. 5 in Fig. 1).
Material deposited: Photosyntype of sporulated oocysts in USNPC No. 88158. Symbiotype *Marmosops dorothea* in the MSB, UNM No. 58512 (NK 15125, female), J. Salazar-Bravo No. JSB-84, 22 July 1987.

3.3.4. Site of infection
Unknown. Oocysts collected from faeces.

3.3.5. Prevalence
Marmosops dorothea: 2/9 (22%) in Santa Cruz.

3.3.6. Etymology
The nomen triviale is derived from the generic part of the scientific name of the host, in the genitive singular ending, meaning "of *Marmosops*".

A multi-group discriminant analysis on log-ten 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 90.1% of the variation in the data being accounted for in the first canonical variate [16]. A plot of discriminant scores indicates minimum polygons enclosing the spread of individuals for each species (Fig. 14). The canonical analysis indicates that as the lengths of the oocysts and sporocysts decrease, their widths increase.

4. Discussion

Species of the Order Didelphimorphia (formerly Marsupialia) are found throughout Central and South America; most are omnivorous, nocturnal, and occur in many habitat types [17, 18]. The Didelphidae, commonly known as American opossums, consists of 15 genera and about 63 species [18, 19].

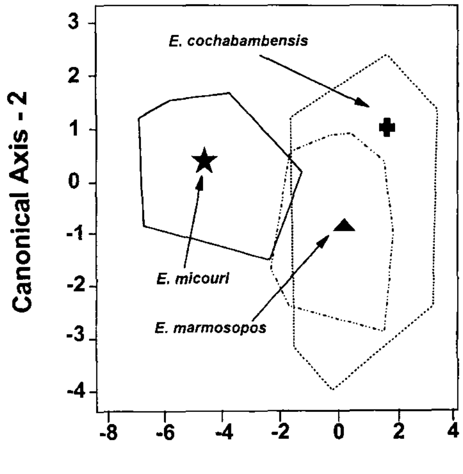

Fig. 14. Plot of discriminant scores of mensural data for the three *Eimeria* spp. named. The symbols on the plot represent the multivariate means (centrioles) of the three new *Eimeria* spp.

Our knowledge about the phylogenetic and biogeographic relationships among members of the Didelphidae is in a state of flux, as more data on distribution, evolution and ecology are published [17–24]. For instance, we now know that *Thylamys elegans* Waterhouse is restricted to Chile, that *T. venustus* has been distinguished as a separate species based on geographic location and DNA-hybridisation [20, 23], and that *M. c. constantiae* has been given priority over its synonyms, *M. demerarae* and *Marmosa cinerea* [18, 19]. Also, many Bolivian marsupial species now have been given subspecies distinction [19]. Thus, even though our knowledge about the species of South American marsupials, and their relationships to each other, is increasing, we still know very little about the parasites they have and/or share.

In the life-cycle of a coccidium, the oocyst is a resistant stage that leaves the host, usually in the faeces, and is the stage most easily collected and studied. As a result, about 98% of the *Eimeria* species described from mammals are known only from this one life-cycle stage [25]. Because qualitative and quantitative features of sporulated oocysts can vary considerably from host to host, oocyst structure has been used historically to distinguish between and designate new species. Unfortunately, the number of taxonomically useful structural characters is reasonably small (~20) and when oocysts from familial host species look similar, it cannot be said for certain whether or not they are the same or different species. In certain host groups, such as Sciuridae (squirrels), cross-transmission and other evidence suggests that coccidia can be shared between host genera [26], whereas in other groups of mammals (Muridae), DNA and cross-transmission evidence (Hnida and Duszynski, unpublished) suggests that similar-looking oocysts from different hosts will seldom cross generic boundaries [25]. Because very little is known not only about what species are found in South American marsupials, but also about the relationship(s) these parasites have with their natural host species, it is unclear to what extent *Eimeria* spp. from Bolivian marsupials are generalists or host specific. *Eimeria cochabambensis* was recorded in three host species from three genera. The structural and mensural difference of oocysts in these three host species is not sufficient to distinguish each as a separate species and may be due to polymorphism as seen in some members of the Eimeriidae Table 2 [27, 28]. Molecular and cross-transmission studies will be needed to definitively answer these questions.

Among the three new *Eimeria* spp., there was more overlap in the discriminant scores for sporulated oocysts of *E. cochabambensis* and *E. marmosopos* than with either of these species and *E. micouri* Fig. 14. This shows that the oocysts and sporocysts of *E. cochabambensis* and *E. marmosopos* have similar sizes and shapes, and that these shapes change in similar ways. Qualitatively, the sporulated oocysts of *E. cochabambensis* have a two-layered wall, fusiform sporocysts and no substieda body; *E. marmosopos* have a one-layered wall, ovoidal sporocysts with a prominent substieda body which distinguishes them as separate species.

In their work with coccidia of insectivores, Duszynski and Moore [29] suggested that *Eimeria* species might be useful to help determine host phylogeny for at least some host groups. If they are correct, we might predict that *Thylamys*, *Marmosops* and *Monodelphis* are all related closely, because *E. cochabambensis* was found in all genera. In DNA-hybridisation studies, however, *Thylamys* and *Marmosops* were found to be more closely related to each other than either was to other marsupial genera; similarly, *Monodelphis* and *Micoureus* also are thought to be closely related [20, 23]. Thus, a broader view that includes not only mensural features of sporulated oocysts, but also other relevant data sets (cross-transmission studies, host and/or parasite DNA work) seem to be necessary to better understand the intricacies of the co-evolution of *Eimeria* spp. and their hosts.

The biodiversity of the *Eimeria* in American Didelphidae is far from complete, because very few people have studied these organisms. Prior to this study, only six eimerian species were described from marsupials in all the Americas, five from Brazil and one from the USA, from four Didelphidae hosts. Here we survey 22 additional marsupial species and describe three new *Eimeria* species from Bolivia. Only 38% of the American Didelphidae species have been surveyed for coccidian parasites. An increase in surveys of parasitic protozoa is needed and collaborations between parasitologists and other specialists may be the answer at times of limited funding and of habitat loss [30]. One of the benefits of collaborating is the immediate expertise for the host and parasite which will be important in describing the biodiversity from within these hosts.

In 1988, Bandoni and Duszynski [15] suggested that photomicrographs of sporulated oocysts be archived in accredited parasitology museums as "type" specimens, called phototypes or syntypes, because there were no convenient methods (and still are none) to preserve sporulated oocysts in perpetuity for museum collections. Here, we use the term "photosyntype" which, we believe, more correctly labels the phototypes that we archive, according to the terminology in the International Code of Zoological Nomenclature [31].

Acknowledgements

Our gratitude to all of the students in the Duszynski laboratory for their support during the production of this paper. We appreciate the input of L. Couch and J.A. Hnida during the many revisions. Thank you to L. Couch and X. Zhao for assistance with the photomicroscopy and printing, and to L. Hertel for the line drawings. We thank the students and staff of the Collection Boliviana de Fauna of the Museo National de Historia Natural in La Paz, and the students and staff of the Museo Noel Kempff Mercado in Santa Cruz for their assistance in logistics and collecting while in the field. Ms C. Thomas, Dr S. Bandoni and D.W. Reduker contributed greatly during the initial phases of this work. Thank you to J. Salazar-Bravo and the staff of the MSB for assistance in confirming host species identities. Thank you to the two anonymous reviewers whose comments assisted in making this paper better. This work was supported, in part, by several NSF grants: BSR-8408923 to T.L. Yates, BSR-8612329, BSR-9024816, DEB-9496263 to S.L.G. and DEB-9521687 [PEET] to D.W.D.

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