

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Faculty Publications from the Harold W. Manter  
Laboratory of Parasitology

Parasitology, Harold W. Manter Laboratory of

---

2006

# Seven New species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from Colubrid Snakes of Guatemala and a Discussion of What to Call Ellipsoid Tetrasporocystic, Dizoic Coccidia of Reptiles

I. M. Asmundsson

*United States Department of Agriculture*, [asmundsson@anri.barc.usda.gov](mailto:asmundsson@anri.barc.usda.gov)

Donald W. Duszynski

*University of New Mexico*, [eimeria@unm.edu](mailto:eimeria@unm.edu)

J. A. Campbell

*University of Texas at Arlington*

Follow this and additional works at: <http://digitalcommons.unl.edu/parasitologyfacpubs>



Part of the [Parasitology Commons](#)

---

Asmundsson, I. M.; Duszynski, Donald W.; and Campbell, J. A., "Seven New species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from Colubrid Snakes of Guatemala and a Discussion of What to Call Ellipsoid Tetrasporocystic, Dizoic Coccidia of Reptiles" (2006). *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*. 199.  
<http://digitalcommons.unl.edu/parasitologyfacpubs/199>

This Article is brought to you for free and open access by the Parasitology, Harold W. Manter Laboratory of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications from the Harold W. Manter Laboratory of Parasitology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

## Seven new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from colubrid snakes of Guatemala and a discussion of what to call ellipsoid tetrasporocystic, dizoic coccidia of reptiles

I. M. Asmundsson<sup>1,3,\*</sup>, D. W. Duszynski<sup>1</sup> & J. A. Campbell<sup>2</sup>

<sup>1</sup>Department of Biology, The University of New Mexico, Albuquerque, NM 87131, USA

<sup>2</sup>Department of Biology, The University of Texas at Arlington, UTA Box 19498, Arlington, TX 76019, USA

<sup>3</sup>USDA-ARS, Beltsville Agricultural Research Center-East, Bldg. 1180, 10300 Sunnyside Avenue, Beltsville, MD 20705, USA

Accepted for publication 26th August, 2005

### Abstract

During a survey of Guatemalan herpetofauna in the summers of 1998–2000, 29 presumed new species of *Eimeria* Schneider, 1875 were found, seven of which have a distinct elongate-ellipsoidal shape (L/W ratio  $\geq 1.7$ ) and are described herein. Six of the seven new species are similar in oöcyst length, width and L/W ratio and sporocyst length, width and L/W ratio, lack a micropyle, oöcyst residuum, Stieda body, sub- and parastieda bodies, have a polar granule and sporocyst residuum, and their sporocysts appear to have dehiscence sutures. The seventh is slightly smaller and has sporocysts with a Stieda body. The new species are: *E. coniophanes* n. sp. – whose sporulated oöcysts from *Coniophanes fissidens* are  $29.2 \times 14.9$  ( $27\text{--}31 \times 13\text{--}16$ )  $\mu\text{m}$ , with sporocysts  $10.0 \times 7.8$   $\mu\text{m}$ ; *E. coniophis* n. sp. – from *Conophis lineatus* are  $32.0 \times 16.5$  ( $30\text{--}34 \times 14\text{--}18$ )  $\mu\text{m}$ , with sporocysts  $10.2 \times 8.9$   $\mu\text{m}$ ; *E. dryomarchoni* n. sp. – from *Dryomarchon corais* are  $32.2 \times 17.7$  ( $31\text{--}34 \times 17\text{--}19$ )  $\mu\text{m}$ , with sporocysts  $10.7 \times 8.6$   $\mu\text{m}$ ; *E. leptophis* n. sp. – from *Leptophis mexicanus* are  $29.5 \times 17.0$  ( $28\text{--}31 \times 16\text{--}18$ )  $\mu\text{m}$ , with sporocysts  $10.0 \times 9.1$   $\mu\text{m}$ ; *E. oxybelis* n. sp. – from *Oxybelis aeneus* are  $31.8 \times 16.5$  ( $29\text{--}33 \times 15\text{--}18$ )  $\mu\text{m}$ , with sporocysts  $10.3 \times 8.8$   $\mu\text{m}$ ; and *E. scaphiodontophis* n. sp. – from *Scaphiodontophis annulatus* are  $30.0 \times 15.3$  ( $28\text{--}33 \times 14\text{--}16$ )  $\mu\text{m}$ , with sporocysts  $9.9 \times 7.9$   $\mu\text{m}$ . Sporulated oöcysts of *E. siboni* n. sp. from *Sibon nebulata* are  $24.3 \times 14.2$  ( $21\text{--}27 \times 13\text{--}16$ )  $\mu\text{m}$ , with sporocysts  $10.0 \times 7.1$   $\mu\text{m}$  and with a Stieda body. We conclude that until all aspects of each life-cycle are known, it is prudent at this time to name all tetrasporocystic dizoic coccidia from snakes as members of *Eimeria* rather than place some of them in *Choleoimeria* Paperna & Landsberg, 1989.

### Introduction

Little is known of the biology and diversity of species of *Eimeria* Schneider, 1875 infecting reptiles and amphibians. To date relatively few coccidia (Apicomplexa: Eimeriidae) from these host groups have been described. The scarcity likely reflects their seldom having been looked for by herpetologists and/or parasitologists rather than an inherent scarcity. In the few surveys that have been done, the high prevalence of coccidia

infection found in snakes indicates a significant potential for discovery of numerous new species. Fewer than 60 valid *Eimeria* species (Duszynski & Upton, unpub.) have been described previously from the 2,955 species of snakes (Uetz, 2003). During a survey of the herpetofauna of Guatemala, faecal samples were collected and examined for coccidians to examine the diversity of these parasites found in reptiles and amphibians. Locations for collecting the hosts were chosen to represent many of Guatemala's diverse environments, ranging from high mountains to low tropical rainforests. In the course of our survey, a total of 29 new *Eimeria* species were discovered.

\*Author for correspondence (E-mail: asmundsson@anri.barc.usda.gov)

*E. dermatophis* Asmundsson, Campbell & Duszynski, 2000 was described from *Dermophis mexicanus*, the plumbeous Central American caecilian, two new *Eimeria* species were found in anurans (unpub. data) and four more were found in lizards (unpub. data). The remaining 22 new *Eimeria*-like species were found in snakes. Here we describe seven of these species, all with elongate-ellipsoidal oöcysts and a L/W ratio  $\geq 1.7$ , and all inhabiting members of the family Colubridae, which contains approximately two-thirds of all living snakes.

### Materials and methods

Host snakes were collected by hand in Guatemala during the seasonal rains of 1998–2000, between late May and mid-July. In most cases the hosts were identified to species in the field. They were kept alive temporarily in plastic or cloth bags and stored in coolers, and then catalogued, killed by injection of Nembutol or Chlorotone and dissected for endo- and ectoparasites immediately or within a few days of capture. They were subsequently preserved and deposited in the vertebrate collection at the University of Texas at Arlington. Fresh faeces were removed from the large intestine of each host and placed in separate snap-top glass vials containing 2.5% (w/v) aqueous potassium dichromate ( $K_2Cr_2O_7$ ). In addition, smears were made of the gall-bladder contents for most snakes to determine if *Eimeria* oöcysts were present in the bile. The slides were immediately air-dried, fixed with absolute methanol, and then stained with Giemsa's stain upon return to the laboratory. The faecal samples were processed in the laboratory, and oöcysts were measured and photographed in accordance with the guidelines of Duszynski & Wilber (1997). Standardised abbreviations for oöcyst/sporocyst structures are those used by Wilber et al. (1998), except that we use SZ instead of SP for sporozoite: *oöcyst characters*: length (L), width (W), their range and ratio (L/W), micropyle (M), residuum (OR) and polar granule (PG); *sporocyst characters*: length (L), width (W), their range and ratio (L/W), Stieda body (SB), substieda body (SSB), parastieda body (PSB), residuum (SR), sporozoites (SZ), refractile bodies (RB) and nucleus (N) in SZ. All measurements are in micrometres, with ranges in parentheses following the mean.

### *Eimeria coniophanes* n. sp.

*Type-host*: *Coniophanes fissidens* (Günther).

*Other hosts*: None.

*Type-locality*: Central America: Guatemala, Departamento de San Marcos, Pacific versant on the lower slopes of Volcán Tajumulco, Finca San Ignacio, 14°56.45'N, 92°01.90'W.

*Prevalence*: 2 of 4 (50%).

*Sporulation*: Endogenous; both sporulated and unsporulated oöcysts were found in the gall-bladder contents.

*Site of infection*: Presumably, the epithelium of the gall-bladder and/or bile-duct since oöcysts were collected from gall-bladder (GB) contents and stained GB smears from the two infected snakes had elongate-ellipsoidal oöcyst, while a stained intestinal smear did not.

*Type-material*: Symbiotype host (Frey et al., 1992) deposited in the herpetological collection, University of Texas at Arlington, R-46548. Photosyn-types of sporulated oöcysts deposited in the US National Parasite Collection (USNPC), Beltsville, Maryland, no. 095396.00.

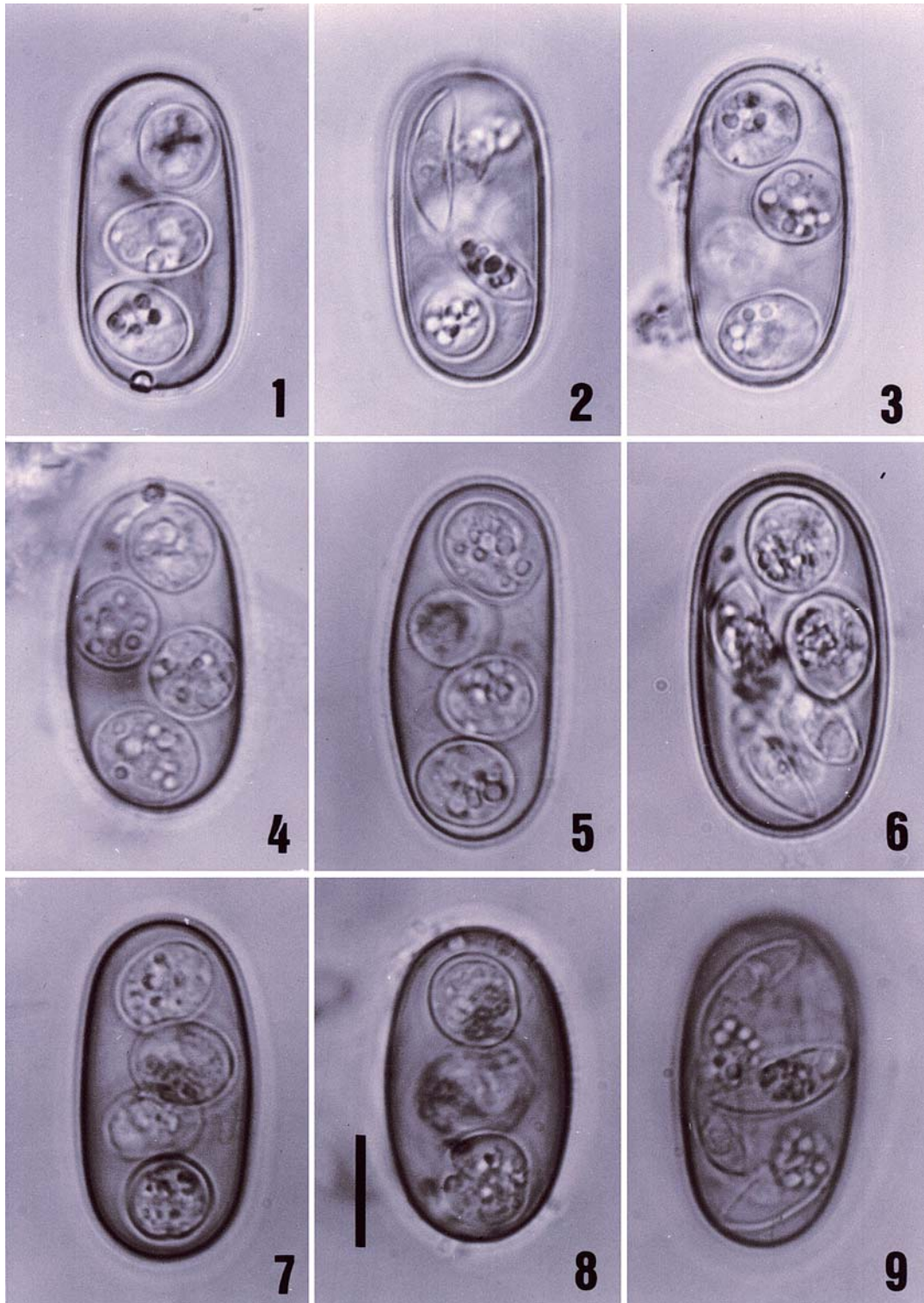
*Description* (Figures 1–3, 19)

*Sporulated oöcyst*. Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness:  $< 1$ ; wall characteristics: appears to be composed of single smooth layer; L $\times$ W (n=50): 29.2 $\times$ 14.9 (27–31 $\times$ 13–16); L/W ratio: 2.0 (1.7–2.2); M and OR: both absent; PG: 1, refractile. Distinct features of oöcyst: PG attached to outer wall of sporocyst or inner wall of oöcyst.

*Sporocysts and sporozoites*. Sporocyst shape: subspheroidal; L $\times$ W (n=45): 10.0 $\times$ 7.8 (9–11 $\times$ 7–8); L/W ratio: 1.3 (1.1–1.4); SB, SSB and PSB: all absent; SR: present; SR characteristics: composed of many medium sized, spheroid globules that obscure SZ; SZ (n=7): banana-shaped, 13.0 $\times$ 4.2 (12–13 $\times$ 4–5) when released from sporocyst; RB: not visible. Distinctive features of sporocyst: sporocysts with longitudinal suture that often ruptures, leaving SZ free within oöcyst.

*Remarks*

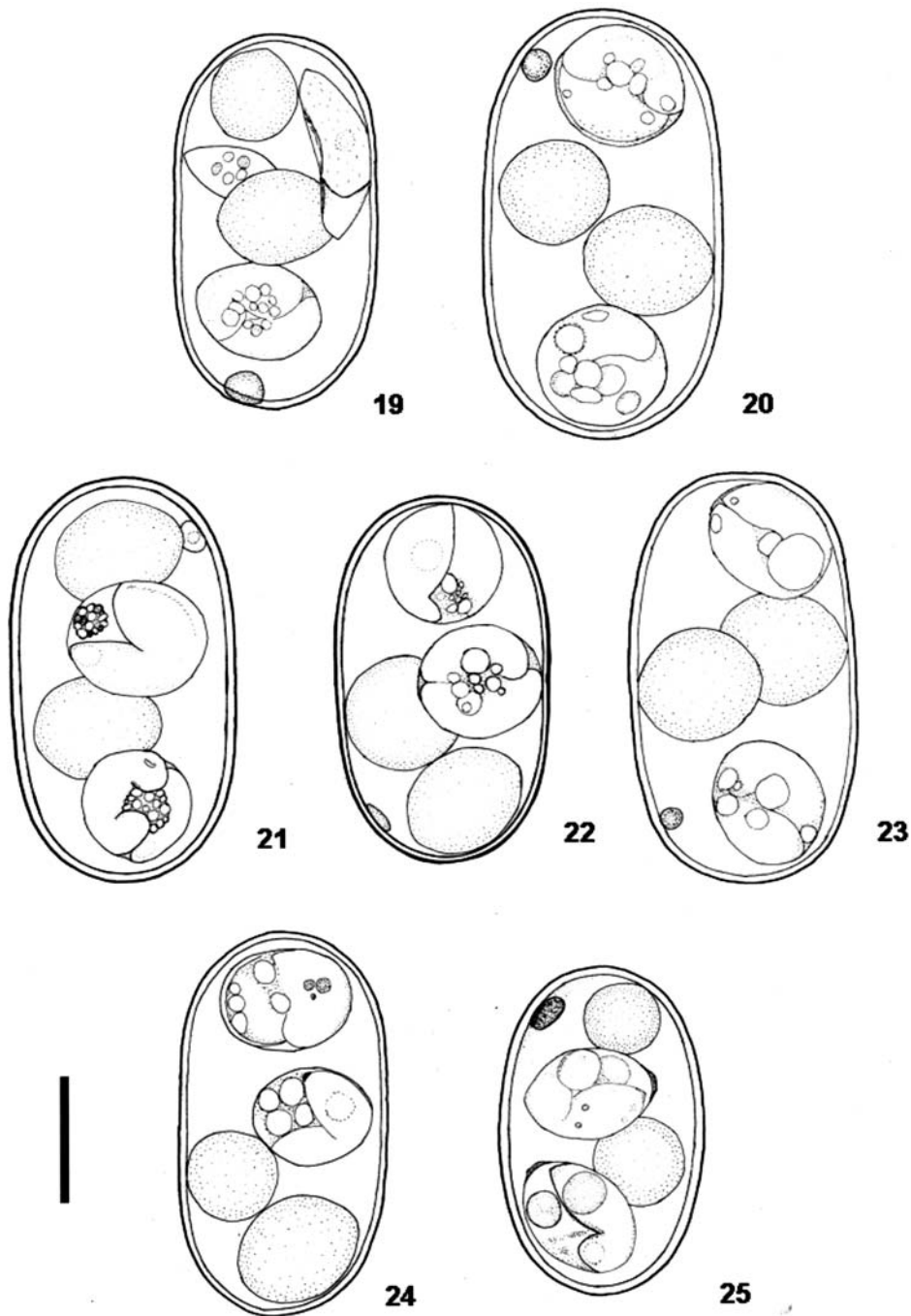
Within the Colubridae there are about 328 genera and 1,876 species Uetz (2003). Only 43 (13%)



Figures 1–9. Photomicrographs of elongate-ellipsoidal sporulated oocysts from colubrid snakes of Guatemala. 1–3. *Eimeria coniophanes* n. sp. 4–6. *E. conophis* n. sp. 7–9. *E. drymarchoni* n. sp. Scale-bar: 10  $\mu$ m.



Figures 10–18. Photomicrographs of elongate-ellipsoidal sporulated oocysts from colubrid snakes of Guatemala. 10–12. *Eimeria leptophis* n. sp. 13–14. *E. oxybelis* n. sp. 15–16. *E. scaphiodontophis* n. sp. 17–18. *E. siboni* n. sp (note SB, arrowed). Scale-bar: 10  $\mu$ m.



Figures 19–25. Line drawings of elongate-ellipsoidal sporulated oocysts from colubrid snakes of Guatemala. 19. *Eimeria coniophanes* n. sp. 20. *E. conophis* n. sp. 21. *E. drymarchoni* n. sp. 22. *E. leptophis* n. sp. 23. *E. oxybelis* n. sp. 24. *E. scaphiodontophis* n. sp. 25. *E. siboni* n. sp. Scale-bar: 10  $\mu$ m.

genera and 88 (<5%) species of colubrids have ever been examined for coccidia prior to this study and about 45 valid eimerians have been named (Duszynski & Upton, unpub.). Of these, there are

nine named *Eimeria* species with elongate-ellipsoidal oocysts of similar size and shape ( $L/W \geq 1.6$ ) to *E. coniophanes*, as well as to the other new species described below. None of these known species

occur in the same snake genera as the species we found. They include *E. zamenis* Phisalix, 1921 in *Coluber constrictor* from Brazil (?) and North America, *E. collanuli* Wacha & Christiansen, 1974 in *Diadophis punctatus* from Iowa, *E. lampropeltis* Anderson, Duszynski & Marquardt, 1968 in *Lampropeltis calligaster* from Illinois, *Eimeria* sp. of Van Peenan & Birdwell (1968) in *Lampropeltis getulus* from California, *E. persica* (Phisalix, 1925) Levine & Becker, 1933 in *Nerodia natrix* from Italy, *E. natrixis* Wacha & Christiansen, 1975 in *Natrix sipedon* from Iowa, *E. dhamini* Mandal & Mukherjee, 1977 in *Ptyas mucosus* from India, *E. papillosum* Upton & McAllister, 1990 in *Salvadora grahamiae* from Texas, and *E. arnaldoi* Pinto & Maciel, 1929 in *Thamnodynastes strigilis* from Brazil.

Sporulated oöcysts of *E. zamenis* lack a PG, which those of *E. coniophanes* possess and its SZ are longer (19 vs 13); otherwise, the oöcysts and sporocysts of the two species are remarkably similar. The major characters suggesting they are distinct are that they are found in different host genera, which are widely separated geographically. Sporulated oöcysts of *E. collanuli* are similar in size to those of *E. coniophanes*, but have an OR which *E. coniophanes* lacks and lack a PG, which *E. coniophanes* has. Oöcysts of *E. lampropeltis* lack a PG, which those of *E. coniophanes* have and the sporocysts of the former are larger, 13.9×7.7 vs 10.0×7.8, and have a larger L/W ratio, 2.0 vs 1.3. Oöcysts of *Eimeria* sp., described but never named by Van Peenan & Birdwell (1968), are much larger, 38.4×20.9, than those of *E. coniophanes*, 29.2×14.9, and have a smaller L/W ratio, 1.8 vs 2.0. Oöcysts of *E. persica* have a very fragile wall that easily distorts when handled and lack a PG, which those of *E. coniophanes* possess; they are also found in different genera on different continents. Oöcysts of *E. natrixis* lack a PG and have large sporocysts (15.7×8.2, L/W 1.9) with a SB, characters that clearly distinguish it from those of *E. coniophanes*. Oöcysts of *E. dhamini* are distinguished from those of *E. coniophanes* by a thick oöcyst wall, c.5, composed of two layers and by lacking a PG. Oöcysts of *E. papillosum* are similar to those of *E. coniophanes* in all metrical and qualitative features except that its oöcyst wall has two obvious layers, the outer of which is covered by numerous papules, which the one-layered wall of *E. coniophanes* lack. Finally, the sporulated oöcysts

of *E. arnaldoi* differ from those of *E. coniophanes* by having two oöcyst walls, spheroid sporocysts (L/W 1.0) and lack a PG vs the single-layered oöcyst wall, subspheroid sporocysts (L/W 1.3) and PG found in *E. coniophanes*.

### *Eimeria conophis* n. sp.

*Type-host*: *Conophis lineatus* (Duméril, Bibron & Duméril).

*Other hosts*: None.

*Type-locality*: Central America: Guatemala, Departamento de Zacapa, San Vicente, Aldea El Arenal.

*Prevalence*: 1 of 4 (25%).

*Sporulation*: Unknown. Oöcyst faecal suspensions were kept in vials of 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution for 2 months at c.24°C prior to discovery and measurement.

*Site of infection*: Unknown. The oöcysts we measured were collected from faeces and one stained GB smear from the infected snake was negative for oöcysts.

*Type-material*: Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-46850. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095397.00.

### *Description* (Figures 4–6, 20)

*Sporulated oöcyst*. Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: c.1; wall characteristics: appears to be composed of single smooth layer; L×W (n=25): 32.0×16.5 (30–34×14–18); L/W ratio: 1.9 (1.7–2.4); M and OR: both absent; PG: 1 highly refractile, spheroid globule that often appears to be touching or attached to outside of sporocyst wall or to inside of oöcyst wall. Distinct features of oöcyst: association of the PG with oöcyst or sporocyst wall.

*Sporocysts and sporozoites*. Sporocyst shape: subspheroidal; L×W (n=25): 10.2×8.9 (9–11×8–10); L/W ratio: 1.2 (1.1–1.3); SB, SSB and PSB: all absent; SR: present; SR characteristics: composed of c.20 small-medium refractile globules; SZ (N=1): 14×4 when released from sporocyst; RB: not visible. Distinctive features of sporocyst: wall with longitudinal sutures, often ruptures leaving SZ loose within oöcyst.

Table 1. Similarity of the metrical data of oöcysts and sporocysts of the seven *Eimeria* species with elongate-ellipsoidal oöcysts found in colubrid snakes from Guatemala. All oöcysts lack a micropyle and oöcyst residuum, all sporocysts lack a sub- and parasitella bodies and all sporocysts have a sporocyst residuum, so these features are omitted from this table.

Eimeria species	Oöcyst				Sporocyst				SB†
	L (range)	W (range)	L/W (range)	PG*	L (range)	W (range)	L/W (range)		
<i>E. contiophanes</i> n. sp. ex <i>Contiophanes fissidens</i>	29.2 (27–31)	14.9 (13–16)	2.0 (1.7–2.2)	1	10.0 (9–11)	7.8 (7–8)	1.3 (1.1–1.4)	–	
<i>E. conophis</i> n. sp. ex <i>Conophis lineatus</i>	32.0 (30–34)	16.5 (14–18)	1.9 (1.7–2.4)	1	10.2 (9–11)	8.9 (8–10)	1.2(1.1–1.3)	–	
<i>E. drymarchoni</i> n. sp. ex <i>Drymarchon corais</i>	32.2 (31–34)	17.7 (17–19)	1.8 (1.7–2.0)	1	10.7 (10–11)	8.6 (8–10)	1.3 (1.1–1.5)	–	
<i>E. leptophis</i> n. sp. ex <i>Leptophis mexicanus</i>	29.5 (28–31)	17.0 (16–18)	1.7 (1.6–1.9)	1	10.0 (9–11)	9.1 (8–10)	1.1 (1.0–1.2)	–	
<i>E. oxybelis</i> n. sp. ex <i>Oxybelis aeneus</i>	31.8 (29–33)	16.5 (15–18)	1.9 (1.6–2.2)	1 +	10.3 (10–11)	8.8 (8–9)	1.2 (1.1–1.4)	–	
<i>E. scaphiodontophis</i> n. sp. ex <i>Scaphiodontophis annulatus</i>	30.0 (28–33)	15.3 (14–16)	2.0 (1.7–2.3)	1	9.9 (8–11)	7.9 (7–8)	1.3 (1.0–1.4)	–	
<i>E. siboni</i> n. sp. ex <i>Sibon nebulata</i>	24.3 (21–27)	14.2 (13–16)	1.7 (1.5–1.9)	1–2	10.0 (7–11)	7.1 (6–8)	1.4 (1.0–1.6)	+	

\* Polar granule

† Stiedea body

### Remarks

This is the first *Eimeria* species described from any species of *Conophis*, but there are four named species from other colubrids with oöcysts that resemble those of *E. conophis* n. sp. in both size and shape. The oöcysts of this species most closely resemble those of *E. contiophanes* n. sp. (see above and Table 1), but are found in a different host genus. They closely resemble those of *E. zamenis*, but have a PG which *E. zamenis* lacks, and are found in a different host genus, which is geographically separated from Brazil (?) and the mid-western U.S., where *E. zamenis* was purportedly found in *Coluber*, *Lampropeltis* and *Masticophis*. They superficially resemble the oöcysts of *E. collanuli*, but these lack a PG and have an OR, while *E. conophis* sporulated oöcysts have a PG but lack an OR. Finally, they closely resemble in size the oöcysts of *E. papillosum*. There are minor differences in the width of their oöcysts and length of their sporocysts, but the most obvious structural difference is the presence of numerous surface papules which oöcysts of *E. conophis* lack. They also are found in different host genera separated by a wide geographical distance.

### *Eimeria drymarchoni* n. sp.

*Type-host*: *Drymarchon corais* (Boie).

*Other hosts*: None.

*Type-locality*: Central America: Guatemala, Departamento de Zacapa, San Vicente, Aldea El Arenal.

*Prevalence*: 2 of 3 (67%).

*Sporulation*: Unknown. Oöcyst faecal suspensions were kept in vials of 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution for 2 months at c.24°C prior to discovery and measurement.

*Site of infection*: Unknown. Oöcysts we measured were collected from faeces. Although one stained GB smear from one infected snake had elongate-ellipsoid structures identical to presumed oöcyst shells seen in the gall-bladder smears from *C. fissidens*, another stained smear from the intestine did not have such structures in it.

*Type-material*: Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-46615. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095398.00.



*Description* (Figures 7–9, 21)

*Sporulated oöcyst.* Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: *c.*1; wall characteristics: appears to be composed of single rough layer; L×W (n = 25): 32.2×17.7 (31–34×17–19); L/W ratio: 1.8 (1.7–2.0); M and OR: both absent; PG: 1 highly refractile, spheroid globule, 1–2×1–2, often appears to be touching or attached to wall of sporocyst. Distinct features of oöcyst: relationship of PG to sporocyst wall.

*Sporocysts and sporozoites.* Sporocyst shape: ellipsoidal; L×W (n = 25): 10.7×8.6 (10–11×8–10); L/W ratio: 1.3 (1.1–1.5); SB, SSB and PSB: all absent; SR: present; SR characteristics: cluster of small to medium globules, which stay together after sporocyst wall breaks; SZ (N = 3): 15×5 when released from sporocyst; RB: not visible. Distinctive features of sporocyst: wall with sutures, often ruptures leaving SZ loose within oöcyst.

*Remarks*

This is the first *Eimeria* species described from any species of *Drymarchon*, but there are seven named species from other colubrids with oöcysts that resemble those of *E. drymarchoni* n. sp. in both size and shape. The oöcysts of this species most closely resemble those of *E. coniophanes* n. sp. and *E. conophis* n. sp. (see above and Table 1), but are found in a different host genus. Sporulated oöcysts of *E. zamenis*, *E. natricis*, *E. collanuli* and *E. lampropeltis* also are similar in most size measurements, but differ from those of *E. drymarchoni* by lacking a PG, those of *E. natricis* and *E. lampropeltis* have much longer sporocysts (L/W 1.9 and 2.0, respectively); those of *E. natricis* have a SB and those of *E. collanuli* have an OR. The other species described from a colubrid snake that is similar in oöcyst size and shape is *E. papillosum*, which differs from *E. drymarchoni* by having a thick, bi-layered wall with papules and smaller SZ, 13.0×2.9 vs 15×5. The host genus and geographical distances further differentiate these last five species from *E. drymarchoni*.

***Eimeria leptophis* n. sp.**

*Type-host:* *Leptophis mexicanus* Duméril, Bibron & Duméril.

*Other hosts:* None.

*Type-locality:* Central America: Guatemala, Departamento de Izabal, Puerto Barrios, Finca El Jabalí, elevation 10 m, 15°44.29'N, 88°20.35'W.

*Prevalence:* 1 of 1 (100%).

*Sporulation:* Unknown. Oöcyst faecal suspensions were kept in vials of 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution for 2 months at *c.*24°C prior to discovery and measurement.

*Site of infection:* Unknown; oöcysts collected from faeces. No gall-bladder smears were made from this animal.

*Type-material:* Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-46689. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095399.00.

*Description* (Figures 10–12, 22)

*Sporulated oöcyst.* Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: *c.*1; wall characteristics: appears to be composed of single smooth layer; L×W (n = 25): 29.5×17.0 (28–31×16–18); L/W ratio: 1.7 (1.6–1.9); M and OR: both absent; PG: 1 highly refractile granule that often appears to be touching or attached to outside of sporocyst wall or to inside of oöcyst wall. Distinct features of oöcyst: PG not visible or absent in *c.*50% of oöcysts.

*Sporocysts and sporozoites.* Sporocyst shape: subspheroidal; L×W (n = 25): 10.0×9.1 (9–11×8–10); L/W ratio: 1.1 (1.0 – 1.2); SB, SSB and PSB: absent; SR: present; SR characteristics: composed of numerous medium-sized refractile globules; RB: not visible. Distinctive features of sporocyst: none.

*Remarks*

This is the first *Eimeria* species described from any species of *Leptophis*, but there are six named species from other colubrids with oöcysts that resemble those of *E. leptophis* n. sp. in both size and shape. The oöcysts of this species most closely resemble those of *E. coniophanes* n. sp., *E. conophis* n. sp. and *E. drymarchoni* n. sp. (see above and Table 1), but are found in a different host genus. There are three other species described from colubrid snakes with similar oöcyst measurements to those of *E. leptophis*. Neither *E. dhamini* nor *E. zamenis* have a PG, which is present in

*E. leptophis*, while the thick (*c.*5), dark oöcyst wall of *E. dhamini* and the papillate outer wall of *E. papillosum* clearly distinguish them from all other elongate-ellipsoidal eimerians from colubrids.

***Eimeria oxybelis* n. sp.**

*Type-host*: *Oxybelis aeneus* (Wagler).

*Other hosts*: None.

*Type-locality*: Central America: Guatemala, Departamento de Zacapa, San Vicente, Aldea El Arenal.

*Prevalence*: 1 of 5 (20%).

*Sporulation*: Unknown. Oöcyst faecal suspensions were kept in vials of 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution for 2 months at *c.*24°C prior to discovery and measurement.

*Site of infection*: Unknown. Oöcysts we measured were collected from faeces. One stained GB smear from the infected snake had elongate-ellipsoidal structures identical to presumed oöcyst shells seen in the gall-bladder smears from *C. fissidens*. However, no complete oöcysts were seen on the slide.

*Type-material*: Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-46847. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095400.00.

*Description* (Figures 13–14, 23)

*Sporulated oöcyst*. Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: *c.*1; wall characteristics: appears to be composed of a single smooth layer; L×W (n=25): 31.8×16.5 (29–33×15–18); L/W ratio: 1.9 (1.6–2.2); M and OR: absent; PG: 1 or more, highly refractile, spheroid globule not seen in all oöcysts, either because it is absent or it may be obscured by sporocysts. Distinct features of oöcyst: PG not visible in all oöcysts.

*Sporocysts and sporozoites*. Sporocyst shape: subspheroidal; L×W (N=25): 10.3×8.8 (10–11×8–9); L/W ratio: 1.2 (1.1 – 1.4); SB, SSB and PSB: all absent; SR: present; SR characteristics: composed of numerous medium-sized refractile globules; SZ (N=1): 14×5 when released from sporocyst; RB: not visible. Distinctive features of sporocyst: wall with longitudinal sutures, often ruptures leaving SZ loose within oöcyst.

*Remarks*

This is the first *Eimeria* species described from any species of *Oxybelis*, but there are 12 named species from other colubrids with oöcysts that resemble those of *E. oxybelis* n. sp. in both size and shape. The oöcysts of this species most closely resemble those of *E. coniophanes* n. sp., *E. conophis* n. sp., *E. drymarchoni* n. sp. and *E. leptophis* n. sp. (see above and Table 1), but are found in a different host genus. Of the eight eimerian species with similar size and shape (see ‘Remarks’ for *E. coniophanes*, above), *E. zamenis*, *E. collanuli*, *E. lampropeltis*, *E. persica*, *E. natricis*, *E. dhamini* and *E. arnaldoi* all lack a PG; *E. dhamini* (thick, dark), *E. papillosum* (surface papules) and *E. persica* (fragile, easily distorted) all have oöcyst walls that distinguish them from *E. oxybelis*; *E. collanuli* has an OR that *E. oxybelis* lacks; and the sporocysts of *E. natricis* have a SB while those of *E. oxybelis* do not. Finally, the latter eight *Eimeria* species are all reported from different genera of colubrids and all are geographically separated from Guatemala by being in the U.S., Europe, India or Brazil. Based on these differences, we believe this to be a distinct species.

***Eimeria scaphiodontophis* n. sp.**

*Type-host*: *Scaphiodontophis annulatus* (Duméril, Bibron & Duméril).

*Other hosts*: None.

*Type-locality*: Central America: Guatemala, Departamento de San Marcos, Pacific versant on the lower slopes of Volcán Tajumulco, Finca San Ignacio, 14°56.45’N, 92°01.90’W.

*Prevalence*: 3 of 3 (100%).

*Sporulation*: Endogenous; both sporulated and unsporulated oöcysts were seen in the gall-bladder contents.

*Site of infection*: Presumably, epithelium of the gall-bladder and/or bile-duct. The oöcysts we measured were collected from the faeces, but stained GB smears from all three snakes had elongate-ellipsoidal oöcysts, while one intestinal smear did not.

*Type-material*: Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-46610. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095401.00.

*Description* (Figures 15–16, 24)

*Sporulated oöcyst.* Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: *c.*1; wall characteristics: appears to be composed of single smooth layer;  $L \times W$  ( $n = 50$ ):  $30.0 \times 15.3$  ( $28\text{--}33 \times 14\text{--}16$ );  $L/W$  ratio: 2.0 (1.7–2.3); *M* and *OR*: both absent; *PG*: 1 refractile body sometimes present and is attached to outside of sporocyst wall or to inside of oöcyst wall. Distinct features of oöcyst: attachment of *PG* to oöcyst or sporocyst wall.

*Sporocysts and sporozoites.* Sporocyst shape: subspheroidal;  $L \times W$  ( $n = 45$ ):  $9.9 \times 7.9$  ( $8\text{--}11 \times 7\text{--}8$ );  $L/W$  ratio: 1.3 (1.0–1.4); *SB*, *SSB* and *PSB*: all absent; *SR*: present; *SR* characteristics: composed of many medium-sized, spheroid globules that obscure *SZ*; *SZ* ( $n = 7$ ): banana-shaped,  $13.0 \times 4.2$  ( $12\text{--}13 \times 4\text{--}5$ ) when released from sporocyst; *RB*: not visible. Distinctive features of sporocyst: they often rupture, leaving *SZ* free within oöcyst.

*Remarks*

This is the first *Eimeria* species described from any species of *Scaphiodontophis*, but there are 13 named species from other colubrids with oöcysts that resemble those of *E. scaphiodontophis* in both size and shape. The sporulated oöcysts of this species most closely resemble those of *E. coniophanes* n. sp., *E. conophis* n. sp., *E. drymarchoni* n. sp., *E. leptophis* n. sp. and *E. oxybelis* n. sp. (see above and Table 1), but are found in a different host genus. And they can be distinguished from the other eight similar species as discussed above (see ‘Remarks’ for *E. coniophanes* and *E. oxybelis*).

***Eimeria siboni* n. sp.**

*Type-host:* *Sibon nebulata* (Linnaeus).

*Other hosts:* None.

*Type-locality:* Central America: Guatemala, Departamento de San Marcos, Pacific versant on the lower slopes of Volcán Tajumulco, Finca San Ignacio,  $14^{\circ}56.45'N$ ,  $92^{\circ}01.90'W$ .

*Prevalence:* 3 of 4 (75%).

*Sporulation:* Unknown. Oöcyst faecal suspensions were kept in vials of 2.5%  $K_2Cr_2O_7$  solution for 2 months at  $c.24^{\circ}C$  prior to discovery and measurement.

*Site of infection:* Unknown. The oöcysts we measured were collected from faeces and one stained GB smear from an infected snake was negative for oöcysts, as was the intestinal smear from the same snake.

*Type-material:* Symbiotype host deposited in the herpetological collection, University of Texas at Arlington, R-45919. Photosyntypes of sporulated oöcysts deposited in the USNPC, no. 095402.00.

*Description* (Figures 17–18, 25)

*Sporulated oöcyst.* Oöcyst shape: elongate-ellipsoidal; number of walls: 1; wall thickness: *c.*1; wall characteristics: appears to be composed of single smooth layer;  $L \times W$  ( $n = 50$ ):  $24.3 \times 14.2$  ( $21\text{--}27 \times 13\text{--}16$ );  $L/W$  ratio: 1.7 (1.5–1.9); *M* and *OR*: both absent; *PG*: 1 large, highly refractile, always present, spheroid to ellipsoidal,  $2 \times 2\text{--}3$ , and occasionally second, small granule next to large body. Distinct features of oöcyst: single large *PG* and occasionally single smaller *PG*.

*Sporocysts and sporozoites.* Sporocyst shape: lemon-shaped;  $L \times W$  ( $n = 50$ ):  $10.0 \times 7.1$  ( $7\text{--}11 \times 6\text{--}8$ );  $L/W$  ratio: 1.4 (1.0–1.6); *SB*: small, knob-like; *SSB* and *PSB*: both absent; *SR*: present; *SR* characteristics: composed of many small to medium globules; *SZ* obscured by *SR*; *RB*: not visible. Distinctive features of sporocyst: *SZ* and *SR* appear to be bound within subspheroid membrane, leaving both ends of sporocyst empty.

*Remarks*

This is the first *Eimeria* species described from any species of *Sibon*, but there are 14 named species from other colubrids with oöcysts that resemble those of *E. siboni* n. sp. in both size and shape. The oöcysts of this species most closely resemble those of *E. coniophanes* n. sp., *E. conophis* n. sp., *E. drymarchoni* n. sp., *E. leptophis* n. sp., *E. oxybelis* n. sp. and *E. scaphiodontophis* n. sp. (see above and Table 1), but, unlike the others, they have sporocysts with an *SB*, which the others lack; they have the smallest ellipsoid oöcysts of any we collected from colubrids in Guatemala, and they also are found in a different host genus. Likewise, they can be distinguished from the other eight species with elongate-ellipsoidal oöcysts discussed

above (see ‘Remarks’ for *E. coniophanes* and *E. oxybelis*) by their smaller size (24.3×14.2) and both the presence of a PG and an SB, a combination all the others lack.

## Discussion

There is very little known about the *Eimeria* species that infect reptiles, as is true of those infecting most host groups. In general, oöcyst morphology both between and within hosts species can be quite diverse, with the only constant feature being their four sporocysts, each with two sporozoites. Molecular evidence indicates that *Eimeria* is paraphyletic (Slapeta et al., 2001; Morrison et al., 2004). This suggests that species lumped within the very large genus *Eimeria* (> 1,500 species to date) should be placed in several different genera.

In 1989, Paperna & Landsberg proposed two new genera, *Acroeimeria* and *Choleoeimeria*, for some of the *Eimeria* and *Eimeria*-like coccidia infecting reptiles, based on the location and development of the endogenous stages and the newly formed oöcyst within the host cell(s). *Acroeimeria* was erected for species that undergo endogenous development in the microvillous zone of intestinal epithelial cells, discharge unsporulated oöcysts that are oval to round, and have sporocysts that lack a Stieda body. *Choleoeimeria* was erected for those *Eimeria*-like coccidia that undergo endogenous development in the epithelium of the gall-bladder, produce oöcysts that are elongate-ellipsoidal (L/W ratio of 1.6–2.2), have endogenous sporulation in the gall-bladder and lumen of the digestive tract, and have sporocysts that are bivalved with a longitudinal suture and lack a Stieda body. Use of these two genera has not gained wide acceptance because we know very little about the endogenous location and development of the vast majority (98%) of named *Eimeria* species. Molecular evidence will help resolve the dilemma of whether or not these proposed genera warrant separate status, but this is not currently available.

*Goussia* Labbé (1896) is another group of *Eimeria*-like oöcysts, found mainly in fish, that also have four sporocysts each with two sporozoites, lack a Stieda body and possessed a longitudinal dehiscence suture. This genus was considered

a synonym of *Eimeria* by most workers (e.g. Pellérdy, 1974) until Dyková & Lom (1981) resurrected it, perhaps justifiably. In their paper, they discussed only fish species, but members of this putative genus also have been described from centipedes, beetles, amphibians and a crocodile (Levine, 1983; Molnár, 1995; Paperna et al., 1997; Gardiner et al., 1986). The oöcysts of *Goussia* species are almost all spheroidal to slightly subspheroidal, with very thin walls, and most are discharged from the host already sporulated (Levine, 1983).

Species of *Choleoeimeria*, along with some reptilian *Eimeria* species, infect bile-duct epithelial cells, have bivalve sporocysts and sporulate endogenously. These characteristics may or may not indicate a separate, distinct lineage from intestinal *Eimeria* species because these features are also shared with *Goussia* species. Species placed in *Choleoeimeria* by Paperna & Landsberg (1989) and Paperna & Lainson (2000) have been described from many families of reptile hosts. Their shared characters indicate the possibility that they are more closely related to each other than to other *Eimeria* sharing the same host species, but probably not more than that. Further complicating this issue is that, even though exogenous sporulation is considered to be a characteristic of *Eimeria*, many exceptions have been reported (*inter alia* Debaissieux, 1914; Guyenot et al., 1922; Pinto, 1928; Ray & Das Gupta, 1936; Van Peenen et al., 1967; Van Peenen & Birdwell, 1968; Anderson et al., 1968; Iskander & Tadros, 1979; Upton & McAllister, 1988).

Here we report seven species of eimerian oöcysts recovered from snakes in Guatemala. All of these species have elongate-ellipsoidal oöcysts with a L/W ratio  $\geq 1.7$ . Thus, they cannot be either *Acroeimeria* or *Goussia* species based just on the shape of the oöcysts alone. The question is, do they belong to *Eimeria* or *Choleoeimeria*?

Jirku et al. (2002), using 18S rDNA sequence, showed that an “unnamed” *Choleoeimeria* species from another colubrid snake, *Spalerosophis diademata*, formed a sister group to eight eimerians from bird and mammals hosts. They argued that *Choleoeimeria* should be considered a distinct genus based on their sequence data, as well as the unique morphological characters noted above (especially sutured sporocysts). Using only this unnamed species and one species of *Goussia*, they

showed these two species formed separate lineages, both from each other and from a lineage comprised of other coccidia with four sporocysts that had Stieda bodies. They concluded that the sporocyst suture may have arisen early in the evolution of eimeriid coccidia, followed by the later evolution of Stieda and substieda bodies.

Unfortunately, nothing is known of the location of endogenous development for most described *Eimeria* species from snakes as well as other animals. Thus, when possible, smears of gall-bladder contents were made of most of the snakes we collected, but correlating these data with the oöcysts we found still do not allow us to make clear cut decisions. We suggest that the location of infection as indicative of evolutionary relationships and as a defining reason for naming a new genus is tenuous based on our current knowledge of the coccidia that infect reptiles, particularly snakes. Thus, until all aspects of each life-cycle are known, we believe it prudent at this time to name all coccidia that have ellipsoid tetrasporocystic, dizoic oöcysts from reptiles as members of *Eimeria* (*sensu lato*). Although, philosophically, we can agree with Jirku et al. (2002) that *Choleoeimeria* may deserve generic status, there are serious practical and logistic problems to be considered and solved before this will be a useful concept. Clearly much work remains to be done to resolve these issues with any degree of certainty.

## Acknowledgments

This work was supported by Survey and Inventory grants (NSF, DEB 9705277, 0102383) to J.A. Campbell, by a PEET grant (NSF, DEB 9521687) to D.W. Duszynski, and a Survey and Inventory grant (NSF, DEB 9505025) to D.W. Duszynski.

## References

- Anderson, D.R., Duszynski, D.W. & Marquardt, W.C. (1968) Three new coccidia (Protozoa: Telosporae) from kingsnakes, *Lampropeltis* spp., in Illinois, with a description of *Eimeria zamensi* Phisalix, 1921. *Journal of Parasitology*, **54**, 577–581.
- Debaisieux, P. (1914) Recherches sur les coccidies IV. *Eimeria cystis-felleae*, nov. spec. *La Cellule*, **29**, 433–449.
- Duszynski, D.W. & Wilber, P.G. (1997) A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology*, **83**, 333–336.
- Dyková, I. & Lom, J. (1981) Fish coccidia: critical notes on life cycles, classification and pathogenicity. *Journal of Fish Disease*, **4**, 487–505.
- Frey, J.K., Yates, T.L., Duszynski, D.W., Gannon, W.L. & Gardner, S.L. (1992) Designation and curatorial management of type host specimens (symbiotype) for new parasite species. *Journal of Parasitology*, **78**, 930–932.
- Gardiner, C.H., Imes, G.D., Jacobson, E.R. & Foggin, C.M. (1986) Sporulated coccidian oöcysts resembling *Goussia Labbé*, 1896, in viscera of Nile crocodiles. *Journal of Wildlife Disease*, **22**, 575–577.
- Guyenot, E., Naville, A. & Ponse, K. (1922) Deux coccidies parasites de *Tropidonotus natrix*, *Eimeria cystis felleae*, et *E. tropidonoti* n. sp. *Revue Suisse Zoologie*, **30**, 115–157.
- Iskander, A.R. & Tadros, G. (1979) *Eimeria samiae* sp. nov. (Eimeriidae: Sporozoa) from the snake *Python reticulata* and its pathogenicity in the intestine. *Bulletin of the Zoological Society of Egypt*, **29**, 66–71.
- Jirku, M., Modry, D., Slapeta, J.R., Koudela, B. & Lukes, J. (2002) The phylogeny of *Goussia* and *Choleoeimeria* (Apicomplexa: Eimeriina) and the evolution of excystation structures in coccidia. *Protist*, **153**, 379–390.
- Levine, N.D. (1983) The genera *Barrouxia*, *Defretinella*, and *Goussia* of the coccidian family Barrouxiidae (Protozoa, Apicomplexa). *Journal of Protozoology*, **30**, 542–547.
- Molnár, K. (1995) Redescription of *Goussia neglecta* n. comb. (Nöller, 1920) (Apicomplexa: Coccidia) and notes on its occurrence in the gut of tadpoles. *Acta Veterinaria Hungarica*, **43**, 269–275.
- Morrison, D.A., Bornstein, S., Thebo, P., Wernery, U., Kinne, J. & Mattsson, J.G. (2004) The current status of the small subunit rRNA phylogeny of the coccidia (Sporozoa). *International Journal for Parasitology*, **34**, 501–514.
- Paperna, I. & Lainson, R. (2000) Ultrastructural study of meronts and gamonts of *Choleoeimeria rochalimai* (Apicomplexa: Eimeriidae) developing in the gall bladder of the gecko *Hemidactylus mabouia* from Brazil. *Folia Parasitologica*, **47**, 91–96.
- Paperna, I. & Landsberg, H. (1989) Description and taxonomic discussion of eimerian coccidia from African and Levantine geckoes. *South African Journal of Zoology*, **24**, 345–355.
- Paperna, I., Ogara, W. & Schein, M. (1997) *Goussia hyperolisi* n. sp.: a coccidian infection in reed frog *Hyperolis viridiflavus* tadpoles which expires towards metamorphosis. *Diseases of Aquatic Organisms*, **31**, 79–88.
- Pellérdy, L.P. (1974) *Coccidia and coccidiosis*, 2nd ed., Berlin: Paul Parey, 959 pp.
- Pinto, C. (1928) *Eimeria amarali* n. sp. parasita de *Bothrops neuwiedii*, ophideo do Brazil. *Boletim Biologico*, **11**, 11–12.
- Ray, H. & Das Gupta, M. (1936) On an *Eimeria* from *Naja naja*. *Proceedings of the Indian Science Congress*, **23**, 345–346.
- Slapeta, J.R., Modry, M., Votypka, J., Jirku, M., Obornik, M., Lukes, J. & Koudela, B. (2001) *Eimeria telekii* n.sp. (Apicomplexa: Coccidia) from *Lemniscomys striatus* (Rodentia: Muridae): morphology, pathology and phylogeny. *Parasitology*, **122**, 133–143.
- Uetz P. (2003) *EMBL reptile database*. <http://www.embl-heidelberg.de/~uetz/db-info/SpeciesStat.html>.
- Upton, S.J. & McAllister, C.T. (1988) Three new species of *Eimeria* (Apicomplexa: Eimeriidae) from *Nerodia rhombifera* (Serpentes: Colubridae) from Texas. *Journal of Protozoology*, **35**, 511–513.

- Van Peenan, P.F.D. & Birdwell, T.L. (1968) Coccidian parasites of the California banded king snake, *Lampropeltis getulus californiae*. *Parasitology*, **58**, 349–354.
- Van Peenan, P.F.D., Ryan, P.F. & McIntyre, T.J. (1967) *Eimeria korros* and *E. modesta* spp. n. (Protozoa: Eimeriidae) from a snake and a tree shrew in South Vietnam. *Journal of Parasitology*, **53**, 1148–1150.
- Wilber, P.G., Duszynski, D.W., Upton, S.J., Seville, R.S. & Corliss, J.O. (1998) A revision of the taxonomy and nomenclature of the *Eimeria* spp. (Apicomplexa: Eimeriidae) from rodents in the tribe Marmotini (Sciuridae). *Systematic Parasitology*, **39**, 113–135.