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A new eimerian (Apicomplexa: Eimeriidae) from southern short-tailed shrews, *Blarina carolinensis* (Bachman) (Soricimorpha: Soricidae: Soricinae) from southeastern Oklahoma, USA

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Abstract A new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) is described from faecal samples of two of three southern short-tailed shrews, *Blarina carolinensis* (Bachman) (Soricidae) from southeastern Oklahoma, USA. Oöcysts of *Eimeria tkachi* n. sp. are subspheroidal to ovoidal with a rough-pitted, tan colored, bi-layered wall, measure 16.5 × 15.2 µm, and have a length/width (L/W) ratio of 1.1; both micropyle and oöcyst residuum are absent, but polar granule(s) are present. Sporocysts are ovoidal, 9.5 × 6.5 µm, L/W 1.4; a distinct button-like Stieda body is present, but the sub-Stieda and para-Stieda bodies are absent and the sporocyst residuum is composed of large globules distributed throughout the sporocyst. Sporozoites have a spheroidal anterior refractile body, a subspheroidal posterior refractile body, and one centrally-located nucleus. This is the smallest eimerian described thus far from the Soricidae, the initial description of a coccidian from *B. carolinensis*, and the first from any shrew from Oklahoma.

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Introduction

The state of Oklahoma is thought to support at least five species of shrews, including southern short-tailed shrew, *Blarina carolinensis* (Bachman), Elliot's short-tailed shrew, *B. hylophaga* (Elliot), least shrew, *Cryptotis parva* (Say), desert shrew, *Notiosorex crawfordi* (Coues), and southeastern shrew, *Sorex longirostris* Bachman (see Taylor & Wilkinson, 1988; Caire et al., 1989). However, Pfau et al. (2011) suggested that the northern short-tailed shrew, *B. brevicauda* (Say) could occur in eastern Oklahoma. One of these, *B. carolinensis* ranges from southern Illinois to the Gulf of Mexico and from eastern Texas to the Atlantic Ocean (Reid, 2006). In Oklahoma, *B. carolinensis* is known only from Le Flore and McCurtain counties (Caire et al., 1989; Braun et al., 2011). It is listed as vulnerable (S3) in the state by NatureServe (NatureServe Explorer, 2015).

A great deal of information is available on the natural history and ecology of *B. carolinensis*, including information on its helminth parasites (McCay, 2001). Although there are reports of coccidians from numerous other North American shrew species (Duszynski & Upton, 2000), nothing, to date, has been published on

coccidia from *B. carolinensis*. The present study provides a description of an eimerian infecting *B. carolinensis* in southeastern Oklahoma, USA.

Materials and methods

During November 2016, three adult *B. carolinensis* were collected by hand or with Victor® snap-traps from Hochatown, McCurtain County, Oklahoma. Fresh fecal samples were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were initially examined for coccidia after flotation in Sheather's sugar solution (specific gravity 1.30) and two were found to contain numerous unsporulated oöcysts. Specimens were then placed in individual Petri dishes containing a small layer of $K_2Cr_2O_7$ and allowed to sporulate for one week at room temperature (*c.* 23°C). Samples were further examined after flotation using an Olympus BX53 light microscope. Measurements were taken on 42 sporulated oöcysts from a single shrew using Olympus© cellSens 1.14 digital software and reported in micrometers (µm) with the ranges followed by the means in parentheses; photographs were taken on 49 sporulated oöcysts using Nomarski interference-contrast optics. Oöcysts of the new species were *c.*37 days old when measured and photographed. Host vouchers (alcohol-fixed specimens) were accessioned into the Henderson State University Collection, Arkadelphia, Arkansas, USA. Photosyntypes of sporulated oöcysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, USA.

Results

Two of the three (67%) *B. carolinensis* were found to be passing coccidian oöcysts with an eimerian that we describe here as new.

Family Eimeriidae Minchin, 1903
Genus *Eimeria* Schneider, 1875

Eimeria tkachi n. sp.

Type-host: Southern short-tailed shrew, *Blarina carolinensis* (Bachman, 1837) (Mammalia: Soricomorpha: Soricidae) (adult male, voucher HSU 953, collected 29.ix.2016).

Type-locality: Halibut Bay Road, Hochatown (34°09′55.152″N, 94°45′35.8776″W), McCurtain County, Oklahoma, USA.

Type-material: Photosyntypes of sporulated oöcysts are deposited as HWML 103064.

Prevalence: In 2 of 3 (67%).

Sporulation time: Oöcysts were completely sporulated within 7 days at 23°C.

Site of infection: Unknown; oöcysts recovered from faeces.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Eimeria tkachi* n. sp. is urn:lsid:zoobank.org:act:3D6BDAA5-8334-4EF1-9A52-2E22F5313106.

Etymology: The specific epithet is given in honor of Dr Vasyl V. Tkach (University of North Dakota, Grand Forks, North Dakota, USA) for his work on the parasites of North American shrews.

Description (Figs. 1, 2A–D)

Sporulated oöcyst

Oöcyst (n = 42) tan, rough-pitted wall, subspheroidal to ovoidal; 14–19 × 13–18 (16.5 × 15.2), length/width (L/W) ratio 1.0–1.2 (1.1). Wall bi-layered, *c.*1.2

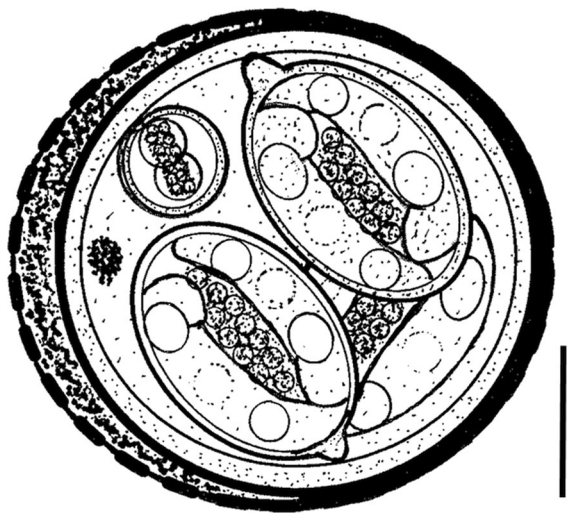

Fig. 1 Schematic line drawing of *Eimeria tkachi* n. sp. ex *Blarina carolinensis*. *Scale-bar*: 5 µm

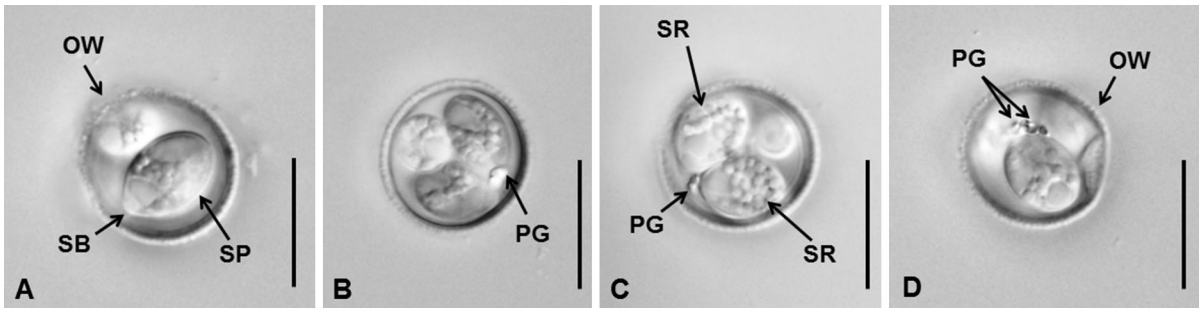

Fig. 2 Nomarski-interference contrast microscopy of sporulated oöcysts of *Eimeria tkachi* n. sp. ex *Blarina carolinensis* (A–D). *Abbreviations*: OW, oöcyst wall; PG, polar granule(s); SP, sporocyst; SR, sporocyst residuum; SB Stieda body. *Scale-bars*: 10 μm

thick, inner layer *c.*0.4, outer layer *c.*0.8. Micropyle and oöcyst residuum absent, polar granule(s) present.

Sporocyst

Sporocyst (n = 42) four, colourless, smooth, ovoidal 8–11 × 6–7 (9.5 × 6.5); L/W ratio 1.3–1.6 (1.4); wall single-layered, *c.*0.4 thick. Distinct button-like Stieda body present, sub-Stieda and para-Stieda bodies absent; sporocyst residuum formed by large globules distributed throughout the sporocyst.

Sporozoites

Sporozoites (not measured) two, sausage-shaped; with a spheroidal anterior refractile body and subspheroidal posterior refractile body and one centrally located nucleus.

Remarks

We limit the comparison of our new form only to those eimerian species from North American shrews of the family Soricidae (see Duszynski & Upton, 2000) (Table 1). When compared to the oöcysts and sporocysts of both *Eimeria blarinae* Todd, French & Levine, 1986 from *B. brevicauda* from Illinois, and *Eimeria brevicauda* Hertel & Duszynski, 1987 from *B. brevicauda* from Ohio (Todd et al., 1986; Hertel & Duszynski, 1987), oöcysts and sporocysts of the new species are significantly smaller. In addition to size differences, *E. brevicauda* possesses a sub-Stieda body (unlike the new species), and *E. blarinae* supposedly has an oöcyst residuum (unlike the new species) but no polar granules (as originally described). However, Duszynski & Upton (2000) mentioned that there was some confusion about the internal structure of oöcysts of *E. blarinae* described by Todd et al. (1986). These authors did not distinguish between polar granules and an oöcyst residuum in *E. blarinae*, the latter internal structure of which was thought to be present as "composed of clear homogenous globules of two sizes." So we are left wondering if *E. blarinae* indeed does possess an oöcyst residuum or actually what would be referred to as polar granules. In addition, the line drawing of *E. blarinae* does not distinguish between these structures, there are no photomicrographs of the species, and it hasn't been reported in another shrew since its original description over 30 years ago.

The new species is most similar in size to *Eimeria cryptotis* McAllister & Upton, 1989 and *Eimeria whitakeri* Upton & McAllister, 1991 from *C. parva* in Texas (Table 1) but both have prominent sub-Stieda bodies (McAllister & Upton, 1989; Upton & McAllister, 1991). In addition, *E. beringiacea* Lynch & Duszynski, 2008 from *Sorex tundrensis* Merriam from Alaska, USA, and Siberia, Russia, is also similar in size to the new species but it possesses a sub-Stieda body and does not have a polar granule (Lynch & Duszynski, 2008). Compared to all the eimerians known from shrews (Duszynski & Upton, 2000; Lynch & Duszynski, 2008), the new species represents the smallest species in the genus.

Based on the differences in morphological and morphometric characteristics noted in the present study, *E. tkachi* is considered a species new to science. Moreover, it is the first eimerian reported from the southern short-tailed shrew as well as the only species reported from Oklahoma shrews.

Table 1 Comparison of the sporulated oöcysts of eimerians from North American shrews

Eimeria spp.	Type-host (current name) Type-locality (others)	Oöcyst shape, size, features[a,b]	Sporocyst shape, size, features[a,b]	Reference
E. beringiacea Lynch & Duszynski, 2008	*Sorex tundrensis* Merriam Alaska, USA (Siberia, Russia)	Subspheroidal 17.7 × 15.6; L/W 1.1 14–24 × 13–20 M, OR, PG: both – SB, SSB, SR: all + PSB: –	Ellipsoidal 10.3 × 6.1; L/W 1.7 7–14 × 4–8	Lynch & Duszynski (2008)
E. blarinae Todd, French & Levine, 1986	*Blarina brevicauda* (Say) Illinois, USA	Subspheroidal to ellipsoidal 21.3 × 18.4; L/W 1.2 18–23 × 15–20 M, PG: both – OR: + SB, SR: both + SSB, PSB: both –	Elongate-piriform 13.2 × 6.7; L/W 2.0 12–14 × 6–8	Todd et al. (1986)
E. brevicauda Hertel & Duszynski, 1987	*B. brevicauda* Ohio, USA (Wyoming, USA)	Spheroidal to subspheroidal 20.3 × 19.7; L/W 1.0 18–23 × 17–23 M, OR: both – PG: + SB, SSB, SR: all + PSB: –	Ovoidal 11.3 × 7.6; L/W 1.5 10–14 × 7–8	Hertel & Duszynski (1987a)
E. cryptotis McAllister & Upton, 1989	*Cryptotis parva* (Say) Texas, USA	Subspheroidal 16.4 × 15.3; L/W 1.1 14–18 × 13–17 M, OR: both – PG: + SB, SSB, SR: all + PSB: –	Ovoidal 10.6 × 7.0; L/W 1.5 9–11 × 6–8	McAllister & Upton (1989)
E. fumeus Hertel & Duszynski, 1987	*Sorex fumeus* Miller Vermont, USA (Massachusetts, New Mexico, Oregon, Vermont, USA; Japan)	Ellipsoidal 27.6 × 22.4; L/W 1.2 25–32 × 20–25 M, OR: both – PG: + SB, SSB, PSB, SR: all +	Football-shaped 15.1 × 8.8; L/W 1.7 13–17 × 8–12	Hertel & Duszynski (1987a)
E. inyoni Hertel & Duszynski, 1987	*Sorex tenellus* Merriam California, USA	Subspheroidal 21.6 × 19.3; L/W 1.1 19–24 × 17–23 M, OR: both – PG: + SB, SSB, SR: all + PSB: –	Ovoidal 11.9 × 7.4; L/W 1.6 10–14 × 6–9	Hertel & Duszynski (1987a)
E. longirostris Hertel & Duszynski, 1987	*S. tenellus* New Mexico, USA (Arizona, California, Colorado, Florida, Massachusetts, Minnesota, Oregon, Pennsylvania, Vermont, Virginia, Washington, Wyoming, USA: Canada)	Spheroidal to subspheroidal 17.1 × 16.6; L/W 1.0 12–22 × 12–21 M, OR: both – PG: + SB, SR: both + SSB, PSB: both –	Ovoidal 10.8 × 6.5; L/W 1.7 6–14 × 4–8	Hertel & Duszynski (1987b)

Table 1 continued

Eimeria spp.	Type-host (current name) Type-locality (others)	Oöcyst shape, size, features[a,b]	Sporocyst shape, size, features[a,b]	Reference
E. soricis Henry, 1932	*S. ornatus* Merriam California, USA (Lithuania, Russia)	Subspheroidal to ellipsoidal 19 × 14; L/W 1.4 19–22 × 13–14 M, OR: both – PG: +	Elongate-ellipsoidal Unknown SB, SSB, PSB: all – SR: +	Henry (1932)
E. tkachi McAllister & Seville, 2017	*Blarina carolinensis* (Bachman) Oklahoma, USA	Subspheroidal to ovoidal 16.5 × 15.2; L/W 1.1 14–19 × 13–18 M, OR: both – PG: +	Ovoidal 9.5 × 6.5; L/W 1.4 8–11 × 6–7 SB, SR: both + SSB, PSB: both –	This study
E. tundraensis Lynch & Duszynski, 2008	*S. tundrensis* Alaska, USA	Spheroidal to subspheroidal 24.8 × 23.5; L/W 1.1 23–26 × 22–25 M, OR: both – PG: +	Elongate-ellipsoidal 15.4 × 8.3; L/W 1.9 12–17 × 7–9 SB, SSB, SR: all + PSB: –	Lynch & Duszynski (2008)
E. vagrantis Hertel & Duszynski, 1987	*Sorex monticolus* Merriam New Mexico, USA (Arizona, Oregon, Vermont, Washington, USA)	Ellipsoidal 26.6 × 21.8; L/W 1.2 24–32 × 20–35 M, OR, PG: all –	Ovoidal 15.9 × 9.0; L/W 1.8 13–17 × 7–10 SB, SSB, SR: all + PSB: –	Hertel & Duszynski (1987a)
E. whitakeri Upton & McAllister, 1991	*C. parva* Texas, USA	Spheroidal to subspheroidal 17.4 × 16.4; L/W 1.1 15–19 × 13–19 M, OR: both – PG: +	Ovoidal 11.2 × 7.5; L/W 1.5 10–12 × 7–8 SB, SSB, SR: all + PSB: –	Upton & McAllister (1991)

[a] Measurements in μm

[b] Descriptions of oöcysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oöcyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oöcyst residuum (OR), polar granule(s) (PG), sporocyst (SP) length (L) and width (W), their ratio (L/W), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), and sporocyst residuum (SR)

Discussion

It has been more than a decade and a half since Duszynski & Upton (2000) summarized the coccidians of Soricimorphs (formerly Insectivora) of the world. Since then, Milek & Seville (2003) reported coccidians from shrews from Wyoming, and Lynch & Duszynski (2008) described two species and added others from shrews in Alaska and Siberia, Russia. Here, we provide an update with what we believe to be 12 species known from North American soricid host species (Table 1).

Duszynski & Upton (2000) listed 23 species of shrews within six genera that have served as hosts for various coccidians (both isosporans and eimerians). However, with over 300 species within 23 genera, that

number represents only 8% of all soricid shrews reported to have coccidians (see Bray & Stockley, 2005). In addition, previous examination of the faeces of 14 additional *B. carolinensis* from two counties (Conway and Union) in Arkansas between July 2012 and April 2013 failed to find any to be passing coccidian oöcysts, so the prevalence of infection with coccidia may be quite low among some shrew populations. Obviously, many species are yet to be examined and future surveys will surely increase our knowledge of shrew coccidians by reporting new host and geographic distributional records, as well as including the possibility of discovering additional species new to science.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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