
Parasites (Coccidia, Trematoda, Acari) of Tricolored Bats, *Perimyotis subflavus* (Chiroptera: Vespertilionidae): New Geographical Records for Oklahoma

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Abstract: The tricolored bat, *Perimyotis subflavus* has been the subject of several surveys on parasites from various parts of its range. However, few populations have been studied west of the Mississippi River and there are apparently no reports of parasites from this bat in Oklahoma. On examination of two *P. subflavus* from a cave near Flint, Delaware County, we found coccidia, *Eimeria macyi*, digenean trematodes, *Ochoterenatrema breckenridgei*, and chiggers, *Perissopalla flagellisetula*. Although these parasites have been reported previously from *P. subflavus* from various locales in other states, all three are reported as new state records from Oklahoma. In addition, we provide, for the first time, photomicrographs of endogenous stages of *E. macyi* as well as a summation of *O. breckenridgei* records from bats of the Western Hemisphere. ©2016 Oklahoma Academy of Science

Introduction

The tricolored bat (formerly, eastern

pipistrelle), *Perimyotis subflavus* F. Cuvier is a small vespertilionid bat that occurs in forested regions from Nova Scotia to Minnesota and southward to the Yucatán Peninsula (Reid 2006).

In Oklahoma, *P. subflavus* is very abundant in the eastern third of the state, but uncommon in the central and western parts (Caire et al. 1989). This bat hibernates in caves or abandoned mines and typically selects a site with high humidity whereas it is mostly found in trees in the warmer months.

Although information is available on parasites of *P. subflavus* from various states (see Fujita and Kunz 1983; Sparks and Choate 2000; Walters et al. 2011), including adjacent Arkansas (McAllister et al. 2004, 2011a, b, 2014), nothing, to our knowledge has been published on any parasites of this bat from Oklahoma. Here, we report new distributional records for three parasites of *P. subflavus* from northeastern Oklahoma.

Methods

Two adult *P. subflavus* were collected by hand from a cave in the vicinity of Flint, Delaware County (36°12'27.83"N, 94°42'15.78"W). They were taken to the laboratory and, following recommendations for care of mammals (Gannon et al. 2007), killed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). The pelage was examined for ectoparasites by brushing the hair over a white enamel pan as well as a superficial examination of the body with a stereomicroscope. Chiggers were collected with fine forceps and placed in a vial containing 70% (v/v) ethanol; they were cleared in lactophenol and slide-mounted in Hoyer's medium (Walters and Krantz 2009). A midventral incision was made from mouth to anus to expose the gastrointestinal tract and fresh feces were collected from the rectum, placed in vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and examined for coccidia by light microscopy using an Olympus BX compound microscope equipped with Nomarski interference-contrast (DIC) optics after flotation in Sheather's sugar solution (sp. gr. 1.20). One positive sample was allowed to complete sporulation in a Petri dish containing a shallow layer of 2.5% $K_2Cr_2O_7$ for five days at room temperature (23 C). Sporulated oocysts were again isolated by

flotation (as above) and were photographed and measured using Olympus Microsuite© software. Measurements were taken on 30 oocysts and reported in micrometers (μm) using a calibrated ocular micrometer and reported in micrometers (μm) with means followed by the ranges in parentheses; photographs were taken using DIC optics. Oocysts were 141 days old when measured and photographed. To examine endogenous stages of the coccidian, tissue from the small intestine was fixed in 10% neutral buffered formalin (NBF) and we used routine histological techniques to prepare them for light microscopy and employed paraffin embedding methods found in Presnell and Schreiber (1997). We dehydrated portions of the intestine and accompanying tissues in a graded series of increasing ethanol solutions (50–100%, v/v), cleared with xylene, and infiltrated and embedded in paraffin wax for 8 hr. We trimmed paraffin/tissue blocks of excess wax, serially sectioned them into ribbons 6 μm thick using a rotary microtome, and affixed sections to microscope slides using Haupt's adhesive while floating on a 2% NBF solution. Tissues were stained using Harris hematoxylin followed by counterstaining with eosin (H & E). For photomicroscopy, we utilized a Nikon Eclipse 600 epi-fluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc., Melville, NY). Trematodes were removed from the intestine and fixed in nearly boiling water without coverslip pressure, placed in 70-95% (v/v) DNA grade ethanol, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada Balsam. Photovouchers of sporulated oocysts and slide-mounted trematodes were accessioned into the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln. Voucher specimens of chiggers are deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia (accession no. L3794). Voucher hosts are deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas as HSU 947–948.

Results and Discussion

Both *P. subflavus* were found to be parasitized, including one harboring a coccidian (HWML 102084) and the other with a digenean trematode (HWML 102051) and a chigger. Data on each species are provided below.

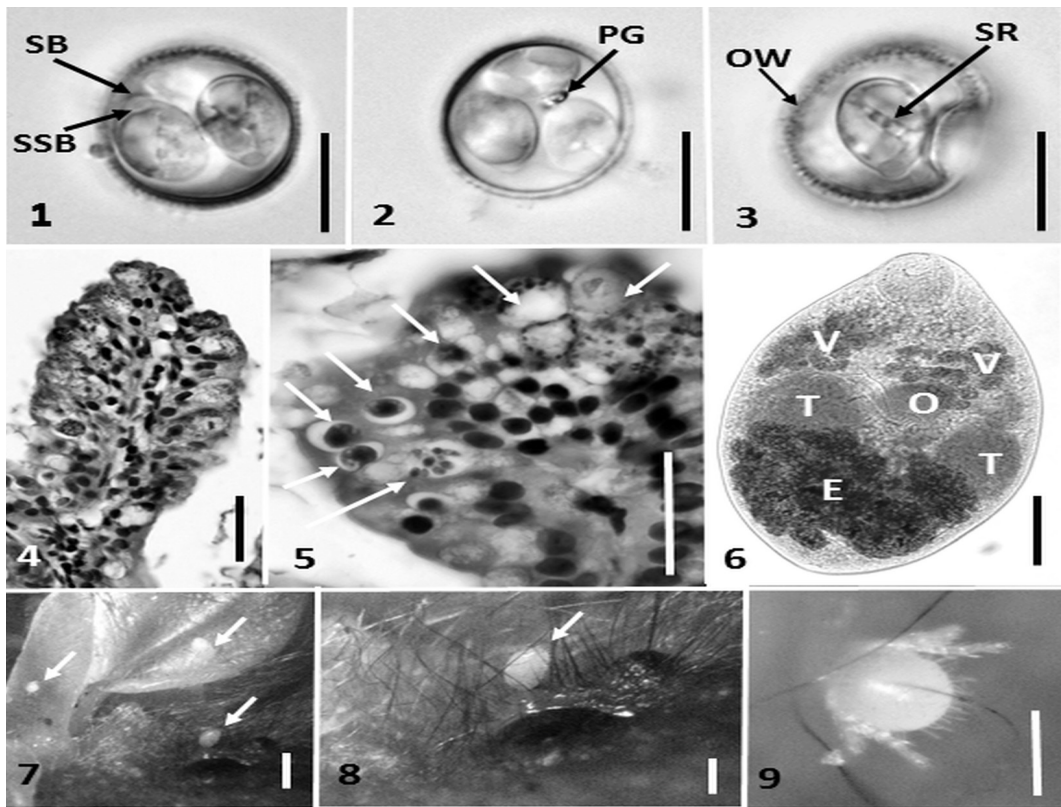
Apicomplexa: Eimeriidae

Eimeria macyi Wheat, 1975 (Figs. 1–5)

One of two *P. subflavus* was found to be passing oocysts of a coccidian that fit the description of *Eimeria macyi* (Wheat 1975; McAllister et al. 2001). This coccidian was originally described from *P. subflavus* from Clarke County,

Alabama (Wheat 1975). McAllister et al. (2001) provided a redescription of *E. macyi* including the first photomicrographs of the coccidian. We report *E. macyi* from Oklahoma for the first time, and it represents only the third coccidian reported from any bat in the state. McAllister et al. (2012) reported *E. catronensis* Scott and Duszynski and *E. tumlisoni* McAllister, Seville and Roehrs from northern long-eared myotis, *Myotis septentrionalis* from Le Flore County.

We also provide, for the first time, photomicrographs of the endogenous development of this coccidian in the small



Figures 1-9. Parasites of *Pipistrellus subflavus* from Oklahoma. 1–3. Sporulated oocysts of *Eimeria macyi*. Abbreviations: OW (oocyst wall); PG (polar granule); SB (Stieda body); SSB (substieda body); SR (sporocyst residuum). Scale bars = 10 μ m. 4. Endogenous stages (H&E) of *Eimeria macyi* in small intestine. Scale bar = 50 μ m. 5. Higher magnification of endogenous stages showing various developmental stages (arrows). Scale bar = 50 μ m. 6. *Ochoterenatrema breckenridgei*. Abbreviations: E (eggs); O (ovary); T (testes); V (vitellaria). Scale bar = 100 μ m. 7. Three *Perissopalla flagellisetula* chiggers, two on right ear, one above right eye (arrows). Scale bar = 2 mm. 8. Close-up view of single chigger above right eye (arrow). Scale bar = 1 mm. 9. Higher magnification of chigger. Scale bar = 1 mm.

intestine which shows several stages (Figs. 4–5). We also report comparative measurements of three isolates of *E. macyi* (Table 1) which shows some variability in the size of oocysts but we believe all represent this coccidian.

Trematoda: Digenea: Lecithodendridae
***Ochoterenatrema breckenridgei* (Fig. 6)**

One of the *P. subflavus* harbored >50 trematodes in the intestine that fit the description of *Ochoterenatrema breckenridgei* (Macy) Lotz and Font (Macy 1936). In the life cycle, anopheline mosquitoes and snails serve as intermediate hosts, and the adult trematode develops in the intestinal tract of bats, which have most likely ingested mosquitoes (Abdel-Azim 1936). This digenean has been reported previously from *P. subflavus* from Arkansas, Indiana, and Minnesota as well as from other bats in five families and various localities (see Table 2). This is the first time *O. breckenridgei* has been reported from Oklahoma.

Acarina: Trombiculidae
***Perissopalla flagellisetula* (Figs. 7–9)**

Six larval chiggers, *Perissopalla flagellisetula* Brennan and White were found attached to the ears on one of two *P. subflavus*. This chigger was originally described from *P. subflavus* from Alabama (Brennan and White 1960). It has not, as far as we can tell, been reported from additional *P. subflavus* or any other host

(Walters et al. 2011). We therefore document a new distributional record for *P. flagellisetula* and only the second time this chigger has been reported since the original description over 46 yr ago. The most frequently recorded chigger from *E. subflavus* is *Euschoengastia pipistrelli* Brennan which has been reported from this host or from other species of bats from the states of Alabama, Arkansas, Illinois, Indiana, Kansas, Kentucky, Missouri, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee and West Virginia (Walters et al. 2011). There is also a record of *Euschoengastia staffordi* Brennan and White from *P. subflavus* in Alabama (Brennan and White 1960). Members of the genus *Euschoengastia* have a pair of distinctive balloon-shaped central scutal setae whereas *P. flagellisetula* has a pair of elongate, plumose central scutal setae.

In summary, little is known about the parasites of Oklahoma bats, and much of what is known concerns their ectoparasites (Reisen et al., 1976; OConner and Reisen 1978, Whitaker et al. 2007), most notably, bat flies from the cave myotis, *Myotis velifer* (Smith 1934; Kessel 1952; Kohls 1954; Zeve 1959, 1960; Caire and Hornuff 1982, 1986; Veal 1983; Caire et al. 1981, 1985). Additional surveys are certainly warranted on parasites of the other 19 species of bats known from the state (Caire et al. 1989) and we expect that additional new host and

Table 1. Comparison of mensural characters of different isolates of *Eimeria macyi* from *Perimyotis subflavus*.

Isolate*	Oocysts (L × W) L/W range	Sporocysts (L × W) L/W range	Reference
Alabama	19.0 × 17.6; 1.1 (16–21 × 15–19); 1.0–1.2	11.0 × 7.0; 1.6 (10–12 × 6–8); 1.5–1.7	Wheat (1975)
Arkansas	22.2 × 20.5; 1.1 (19–25 × 18–24); 1.0–1.2	12.4 × 8.3; 1.5 (11–14 × 7–10); 1.3–1.7	McAllister et al. (2001)
Oklahoma	18.1 × 15.8; 1.1 (16–21 × 13–17); 1.0–1.3	10.1 × 6.4; 1.6 (9–12 × 6–8); 1.5–1.7	This study

*All isolates possessed a polar granule(s), Stieda and substieda bodies, and sporocyst residua without a micropyle and oocyst residuum.

Table 2. Hosts and localities of *Ochoterenatrema breckenridgei*.

Family/Host	Locality	Reference
Molossidae		
<i>Mormopterus minutus</i>	Cuba	Groschaft and Valle, 1969†; Odening, 1969‡; Zdzitowiecki and Rutkowska, 1980‡
<i>Nyctinomops laticaudatus</i>	Cuba	Zdzitowiecki and Rutkowska, 1980‡
<i>Tadarida brasiliensis</i>	Cuba	Zdzitowiecki and Rutkowska, 1980‡
	Florida	Foster and Mertins, 1996
Mormoopidae		
<i>Molossus molossus</i>	Cuba	Odening, 1969‡
	Cuba	Zdzitowiecki and Rutkowska, 1980‡
<i>Mormoops blainvillii</i>	Cuba	Odening, 1969‡; Zdzitowiecki and Rutkowska, 1980‡
Natalidae		
<i>Nyctiellus lepidus</i>	Cuba	Zdzitowiecki and Rutkowska, 1980‡
Phyllostomidae		
<i>Artebius jamaicensis</i>	Cuba	Perez Viguera, 1940*
<i>Phyllonycteris poeyi</i>	Cuba	Groschaft and Valle, 1969†
Vespertilionidae		
<i>Eptesicus fuscus</i>	Cuba	Groschaft and Valle, 1969†; Zdzitowiecki and Rutkowska, 1980‡
	Indiana	Pistole, 1988
	Minnesota	Lotz and Font, 1983, 1985, 1991, 1994
	Wisconsin	Lotz and Font, 1985, 1991, 1994
<i>Myotis keenii</i>	Indiana	Pistole, 1988
<i>Myotis lucifugus</i>	Indiana	Pistole, 1988
<i>Myotis sodalis</i>	Indiana	Pistole, 1988
<i>Perimyotis subflavus</i>	Arkansas	McAllister et al. 2001
	Indiana	Pistole, 1988
	Minnesota	Macy, 1936; Lotz and Font, 1983
	Oklahoma	This study

* Originally reported as *Lecithodendrium pricei*, a synonym of *O. breckenridgei* (per Lotz and Font, 1983).

† Originally reported as *Lecithodendrium vivianae* (fide Odening, 1973).

‡ Originally reported as *O. pricei*, a synonym of *O. breckenridgei* (per Lotz and Font, 1983).

distributional records would be discovered.

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