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Eimeria lancasterensis (Apicomplexa: Eimeriidae), Three Nematodes (Heligmosomoidea: Boehmiellidae, Heligmonellidae), and a Flea (Siphonaptera: Ceratophyllidae) from the Eastern Fox Squirrel, Sciurus niger (Rodentia: Sciuridae) in Arkansas

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Cover Page Footnote

The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM. We thank Drs. S.L. Gardner and G. Racz (HWML) for expert curatorial assistance, J.M. Kinsella (Missoula, MT) for nematode identifications, and L.M. Hardy (Ouachita Mountains Biological Station) for providing gratis housing and laboratory space for CTM. We also thank the Keck DNA Sequencing Facility at Yale University (New Haven, CT) for their assistance with DNA sequencing service.

Eimeria lancasterensis (Apicomplexa: Eimeriidae), Three Nematodes (Heligmosomoidea: Boehmiellidae, Heligmonellidae), and a Flea (Siphonaptera: Ceratophyllidae) from the Eastern Fox Squirrel, *Sciurus niger* (Rodentia: Sciuridae) in Arkansas

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Running Title: Parasites of Eastern Fox Squirrel

Abstract

In Arkansas, the eastern fox squirrel (*Sciurus niger*) is a common inhabitant of the state. Although information is available on ectoparasites of this host in Arkansas, little is known about the endoparasites of this squirrel. A single specimen from Montgomery County was examined and found to harbor the following: a coccidian (*Eimeria lancasterensis*), three nematodes, *Boehmiella wilsoni*, *Citellinema bifurcatum*, and *Sciurodendrium hassalli*, and a flea, *Orchopeas howardi*. We document these nematodes from an Arkansas *S. niger* for the first time, and add mensural and molecular information on *E. lancasterensis* from this host.

Introduction

The eastern fox squirrel, *Sciurus niger* (L., 1758) is the largest tree squirrel in the Western Hemisphere that occurs naturally in temperate forests over most of eastern North America (Hall 1981; Koprowski 1994). In Arkansas, *S. niger* is found statewide (Sealander and Heidt 1990). It inhabits a diversity of deciduous and mixed-forest habitats, but is more common in forest patches (Nixon and Hansen 1987). Fox squirrels feed heavily on tree seeds during much of the year (Koprowski 1994).

Although *S. niger* has been the subject of several studies of its coccidian parasites (Knipling and Becker 1935; Levine and Ivens 1965; Joseph 1972, 1973a, b, 1975; McAllister and Upton 1989; Spurgin and Hnida 2002; Motruik-Smith *et al.* 2009; Ozmen *et al.* 2009), there are no surveys reporting coccidia in any specimen from Arkansas.

Eastern fox squirrels have also been reported to be host of a suite of helminth parasites (Rausch and Tiner 1948; Flyger and Gates 1992). In Arkansas, Davidson (1976) examined some *S. niger* from the Ozarks in Stone County for parasites. There are no other reports of any helminth parasite from this host in the state. Here we report new records for parasites from a *S. niger* from the Ouachitas of Arkansas as well as include additional figures, mensural, and sequence data for a coccidian.

Materials and Methods

On 16 October 2020, an adult squirrel was hit and killed by an automobile on St. Hwy. 8, 3.2 km west of Black Springs, Montgomery County (34° 27' 16.29" N, -93° 46' 20.2872" W). It was opportunistically collected and immediately taken to the lab and processed for parasites. The pelage was brushed over a white enamel tray for ectoparasites. Any found were placed in a vial of 70% (v/v) ethanol and later cleared in 10% (w/v) potassium hydroxide, dehydrated through an ethanol series, further cleared in xylene, and slide-mounted in Canada balsam. A mid-ventral incision was made to expose the viscera and the gastrointestinal (GI) tract from the throat to anus was removed, rinsed in 0.9% (w/v) saline, and organs (including heart, liver, lungs, spleen, and kidneys) were placed in individual Petri dishes. Several 10 cm sections of the GI tract were cut, split lengthwise, and examined under a stereomicroscope for endoparasites. Feces from the rectum was collected and placed in 2.5% (w/v) potassium dichromate. A fecal flotation was accomplished with Sheather's sugar solution (sp. gr. 1.30). Nematodes were examined as temporary mounts

in glycerol.

For analysis of the DNA sequence of the *Eimeria* species, feces in 2.5% (w/v) potassium dichromate was sent to the Fish and Wildlife Disease Laboratory at SUNY-ESF. DNA was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Corp, Irvine, CA) with modifications described in Whipps *et al.* (2020). PCR was performed in 50 µL reaction volumes in Quick-Load® Taq 2X Master Mix (New England Biolabs, Ipswich, MA), 0.25 µM of each primer and 3 µL of template DNA. Overlapping fragments targeting the SSU ribosomal DNA were amplified with primers Eimeria1F (5'-GAT TCA TAG TAA CCG AAC GG) with 18R (Whipps *et al.,* 2003), and Eimeria2F (5'-GGG CAT TCG TAT TTA ACT GTC) with 18R. Amplifications were performed on a C1000™ Thermal Cycler (BioRad Laboratories, Hercules, CA) with initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 56°C for 45 sec, 68°C for 90 sec, and a final extension at 72°C for 7 min. Product amplification was evaluated by observation on a 1% (w/v) agarose gel and the remainder of the sample purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA). DNA was quantified using a DNA spectrophotometer (NanoDrop Technologies Wilmington, Delaware). Sequencing used amplification primers with the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were assembled manually in BioEdit (Hall 1999) and identity analyzed by GenBank BLAST search.

A host photovoucher was deposited in the Eastern Oklahoma State College Collection, Idabel, OK. Voucher specimens of ectoparasites were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, GA. Endoparasites were deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, NE, or samples were retained for molecular analyses.

APICOMPLEXA: EIMERIORINA: EIMERIIDAE

***Eimeria lancasterensis* Joseph, 1969 –** Oocysts (Fig. 1A–C, HWML 216668) of this coccidian were being passed in feces. Oocysts (*n* = 20) were ellipsoidal, 23.5 × 14.3 (18–29 × 11–19) µm, with a length/width ratio (L/W) of 1.6 (1.3–1.8). Bilayered wall was 1.4 (1.1–1.7) with a smooth, occasionally lightly pitted or sculptured, colorless to light yellow outer layer, ~2/3 total thickness; inner layer light yellow.

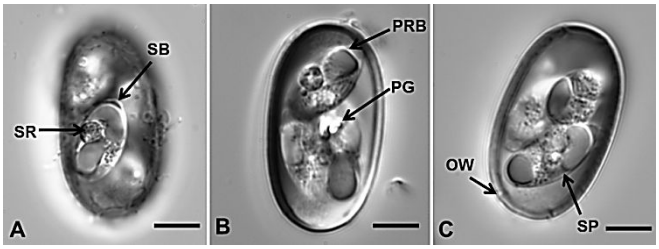

Figure 1. Sporulated oocysts of *Eimeria lancasterensis* from *Sciurus niger* from Montgomery County, Arkansas. A. Stieda body (SB) and sporocyst residuum (SR). B. Polar granule (PG) and posterior refractile body (PRB). C. Oocyst wall (OW) and sporocyst (SP). Scale bars = 10 µm.

Micropyle and oocyst residuum was absent but 1–3 sometimes bilobed polar granule(s) were present. Sporocysts (*n* = 20) were ellipsoidal, (L × W) 11.5 × 6.7 (10–13 × 6–8) µm with an L/W ratio of 1.7 (1.4–2.2). Nipple-like Stieda body was present but subStieda and paraStieda bodies were absent. Sporocyst residuum was composed of various-sized granules forming a compact sphere, or a dense irregular mass located between and across the sporozoites, or a combination of both within the same sporocyst. Sporozoites (not measured) were elongate, anterior end tapered, posterior end rounded with a large, ellipsoidal posterior refractile body. The 1,472 nucleotide SSU DNA sequence for this specimen was submitted to GenBank (accession MZ831509). Our sequence was identical to a sequence from *E. lancasterensis* from eastern gray squirrels*, Sciurus carolinensis* Gmelin in Italy (GenBank accession KT360976) over 1,224 nucleotides.

This is one of the most prevalent coccidians infecting members of the rodent family Sciuridae. It has been reported previously from *S. niger* in Texas (McAllister and Upton 1989a), Nebraska (Spurgin and Hnida 2002), and Virginia and Wyoming (Motriuk-Smith *et al.* 2009). In addition, this coccidian has been reported from *S. carolinensis* from Italy (Hofmannová *et al.* 2016), from a red squirrel, *Sciurus vulgaris* (L.) in Turkey (Ozmen *et al.* 2009) and from *S. carolinensis* in Massachusetts (Joseph 1969, 1972), Florida (Forrester *et al.* 1977), Texas (McAllister and Kessler 2002), and Arkansas (McAllister and Kessler 2002). Although the latter authors reported *E. lancasterensis* in the state from *S. carolinensis*, no mensural data or photomicrographs were provided. Therefore, this is the first report of measurements, accompanying photomicrographs, and molecular data on *E. lancasterensis* from an Arkansas host.

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NEMATODA: HELIGMOSOMOIDEA: BOEHMIELLIDAE

***Boehmiella wilsoni* Lucker, 1943. –** Two specimens (HWML 112234) were found in the stomach. *Boehmiella* spp. are principally characterized by having chitinized sheaths on the lateral and externo-dorsal rays of the bursa. They have short, complex unbranched spicules and females are didelphic. This nematode was described infecting *S. carolinensis* in Florida, Georgia, Minnesota, and West Virginia (Lucker 1943; Rausch and Tiner 1948). Coyner *et al.* 1996) reported it from *S. niger* from Florida, and Whitaker and Mumford (2009) from Indiana. Davidson (1976) reported *B. wilsoni* from *S. carolinensis* from Stone County, Arkansas. More recently, *B. wilsoni* was found in Deppe's squirrel, *Sciurus deppei* Peters in México (Falcon-Ordáz and García-Prieto 2004); in brown agouti, *Dasyprocta variegata* Tschudi in Bolivia (Mollericona *et al.* 2016); and in Ferreira's spiny tree-rat, *Mesomys hispidis* (Desmarest) in Brazil (Andrade-Silva *et al.* 2020). We document *B. wilsoni* in a *S. niger* from Arkansas for the first time.

TRICHOSTRONGYLOIDEA: HELIGMONELLIDAE

***Sciurodendrium hassalli* (Price, 1928). –** Approximately 30 specimens (HWML 112233) were found in the small intestine. Price (1928) originally described this nematode from *S. carolinensis* from Maryland. *Sciurodendrium* spp. are loosely coiled parasites and are characterized by having most of the cuticular ridges discontinuous and scalloped. Species are determined by the pattern of the bursal rays and females are monodelphic. The distribution of *S. hassalli* in sciurids is widespread. Chandler (1942) reported 100% prevalence in fox squirrels from eastern Texas, while Eckerlin (1993) found 50% prevalence in *S. niger* from Maryland and Virginia. It has also been reported from *S. niger* from Florida (Coyner *et al*. 1996), Ohio (Katz 1938) and Tennessee (Reiber and Byrd 1942). Davidson (1976) reported *S. hassalli* from *S. carolinensis* from Stone County. We document *S. hassalli* from an Arkansas eastern fox squirrel for the first time.

***Citellinema bifurcatum* Hall, 1916. –** Two specimens (HWML 112232) were recovered from the small intestine. The type host is the Wyoming ground squirrel, *Urocitellus elegans* (Kennicott) (see Hall 1916). *Citellinema* spp. are tightly coiled parasites characterized by an asymmetrical bursa with a greatly reduced dorsal ray. The spicules are short (380–400 µm) and deeply bifurcated and females are didelphic. It is a common among sciurids where it occurs in squirrels over a range from Colorado, Wyoming, and Saskatchewan, Canada to Maine (Reiber and Byrd 1942). This nematode has also been reported from *S. niger* from Florida (Coyner *et al.* 1996), Indiana (Whitaker and Mumford 2009), Tennessee (Reiber and Byrd 1942), and Ohio (Katz 1938). Davidson (1976) reported *C. bifurcatum* from *S. carolinensis* from Stone County. This nematode is reported from an Arkansas eastern fox squirrel for the first time.

ARTHROPODA: INSECTA: SIPHONAPTERA: CERATOPHYLLIDAE

***Orchopeas howardi* (Baker, 1895). –** a single female (L3851) was recovered. This flea is a common ectoparasite of sciurids, including *S. niger* (Whitaker *et al.* 1976; Lewis 2000). Schiefer and Lancaster (1970) and McAllister *et al.* (2013) reported *O. howardi* previously from *S. niger* from the Arkansas Ozarks. Other hosts from the state include *S. carolinensis*, southern flying squirrel, *Glaucomys volans* (L.), and raccoon, *Procyon lotor* (L.) (McAllister *et al.* 2017). This flea has been reported to transmit North American strains of the causative agent of sporadic epidemic typhus (*Rickettsia prowazekii*), which is maintained enzootically in flying squirrel populations (McDade 1987). Human cases of this disease have been serologically confirmed and recorded in Arkansas (McDade 1987). We report *O. howardi* from a host from the Ouachita uplands of the state for the first time.

In conclusion, we document, for the first time, three nematodes from a *S. niger* from Arkansas. Two of these, *S. hassalli* and *C. bifurcatum*, which have direct life cycles, are proposed to be core species of *S. niger* (Kinsella 1991) and we concur. Although only a single *S. niger* was examined herein it yielded these new records as well as extra mensural and molecular data on the coccidian, *E. lancasterensis*. Additional eastern fox squirrels in Arkansas should be examined for parasites from the southern and eastern parts of its range in the state.

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The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM. We thank Drs. S.L. Gardner and G. Racz (HWML) for expert curatorial assistance, J.M. Kinsella (Missoula, MT) for nematode identifications, and L.M. Hardy (Ouachita Mountains Biological Station) for providing gratis housing and laboratory space for CTM. We also thank

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