

Parasites of Wood Frogs, *Rana sylvatica* (Ranidae), from Arkansas, with a Description of a New Species of *Eimeria* (Apicomplexa: Eimeriidae)

CHRIS T. McALLISTER,¹ STEVE J. UPTON,² STANLEY E. TRAUTH,³ AND CHARLES R. BURSEY⁴

¹ Division of Natural and Applied Sciences, Cedar Valley College, 3030 North Dallas Avenue, Lancaster, Texas 75134-3799,

² Division of Biology, Ackert Hall, Kansas State University, Manhattan, Kansas 66506, e-mail: coccidia@ksuvm.ksu.edu,

³ Department of Biological Sciences, Arkansas State University, State University, Arkansas 72467, e-mail: strauth@navajo.astate.edu, and

⁴ Department of Biology, Pennsylvania State University–Shenango Valley Campus, 147 Shenango Avenue, Sharon, Pennsylvania 16146-1537, e-mail: cxb13@psuvm.psu.edu

ABSTRACT: Thirteen wood frogs, *Rana sylvatica* LeConte, 1825, were collected in February 1994 from Izard County, Arkansas, and examined for parasites. Twelve (92%) were infected with 1 or more parasites, including 8 (62%) with *Opalina* sp., 3 (23%) with *Myxidium serotinum* Kudo and Sprague, 1940, 5 (38%) with unidentified trematode metacercariae, 4 (31%) with *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, 2 (15%) with *Mesocestoides* sp. tetrathyridia, 1 (8%) with *Abbreviata* sp., 1 (8%) with *Oswaldocruzia pipiens* Walton, 1929, and 1 (8%) with *Desserobdella picta* (Verrill, 1872). In addition, 11 (85%) were found to harbor a previously unreported eimerian. Oocysts of *Eimeria fitchi* sp. n. were ovoidal, 21.9×14.3 (20.0 – 24.0×13.2 – 15.2) μm , with a smooth, thin, single-layered wall; shape index (length/width) 1.5 (1.3–1.7). A micropyle, oocyst residuum, and polar granule were absent. The sporocysts were ovoidal, 10.9×7.4 (9.8 – 11.2×7.0 – 8.0) μm ; shape index 1.5 (1.3–1.6). One end of the sporocyst was thickened slightly to form an indistinct Stieda body, and a substieda body was absent. A sporocyst residuum was present, 3.6×1.6 , consisting of large, coarse granules often scattered free among sporozoites. Sporozoites were elongate, 11.1×1.7 (10.4 – 12.0×1.6 – 1.8) in situ, each with 2 refractile bodies. Three new host records are reported for parasites of *R. sylvatica*.

KEY WORDS: *Rana sylvatica*, wood frog, Anura, Ranidae, *Opalina* sp., *Myxidium serotinum*, metacercariae, *Brachycoelium salamandrae*, *Mesocestoides* sp. tetrathyridia, *Abbreviata* sp., *Oswaldocruzia pipiens*, *Desserobdella picta*, *Eimeria fitchi* sp. n.

The wood frog, *Rana sylvatica* LeConte, 1825, is a medium-sized anuran that ranges throughout much of northern North America, from Labrador to Alaska, south and eastward to the southern Appalachians; disjunct populations occur in Newfoundland, Alabama, Arkansas, Colorado, Missouri, North Dakota, and Wyoming (Conant and Collins, 1991). The wood frog is an explosive late winter–early spring breeder in small, fishless, mesic woodland ponds and pools (Johnson, 1987). For most of the year, *R. sylvatica* is secretive and solitary and often difficult to observe among shady ravines, forests near clear streams, leafy pools, cave entrances, and damp wooded hillsides (Johnson, 1987).

Martof (1970) provided a summary of the biology of *R. palustris* in a species account. Walton (1964) provided a summary of the protozoans known to infect *R. sylvatica*, and additional information regarding the parasites of wood frogs is available for individuals from Canada (Staf-

ford, 1905; Fantham et al., 1942; Pearson, 1956; Baker, 1978a, b, 1979a, b; Adamson, 1980; Barta and Desser, 1984; Jones, 1987; Chen and Desser, 1989), Alaska (Metcalf, 1923), Maine (Bouchard, 1951), Maryland (Walton, 1931), Massachusetts (Rankin, 1945), Michigan (Najarian, 1955; Muzzall and Peebles, 1991), New York (Harwood, 1930, 1932), North Carolina (Metcalf, 1923), Ohio (Metcalf, 1923; Odlaug, 1954), and Wisconsin (Williams and Taft, 1980). However, nothing has been published on disjunct populations from the southwesternmost extent of its range in Arkansas. Herein, we provide information on parasites of a small sample of *R. sylvatica* from northern Arkansas, including a description of a new species of *Eimeria*.

Materials and Methods

Thirteen juvenile and adult male *R. sylvatica* ($\bar{x} \pm$ SEM snout–vent length [SVL] = 58.5 ± 1.7 , range 49–69 mm) were collected by hand during breeding activ-

Table 1. Parasites of *Rana sylvatica* from IZARD County, Arkansas.

Parasite	Location in host	Prevalence*
Protozoa		
<i>Eimeria fitchi</i> sp. n.	Intestinal contents, feces	11/13 (85%)
<i>Opalina</i> sp.	Rectum	8/13 (62%)
<i>Myxidium serotinum</i> †	Gall bladder	3/13 (23%)
Trematoda		
Unidentified metacercariae	Mesenteries	5/13 (38%)
<i>Brachycoelium salamandrae</i>	Small intestine	4/13 (31%)
Cestoidea		
<i>Mesocestoides</i> sp.†	Liver, mesenteries	2/13 (15%)
Nematoda		
<i>Abbreviata</i> sp.	Stomach	1/13 (8%)
<i>Oswaldocruzia pipiens</i>	Small intestine	1/13 (8%)
Hirudinea		
<i>Desserobdella picta</i> †	Skin	1/13 (8%)

* Number infected/number examined (percent).

† New host record.

ities in February 1994 from a pond in IZARD County, Arkansas (36°03'N, 91°54'W, elev. 195 m), and examined for parasites. Specimens were placed in plastic bags on ice and returned to the laboratory within 24 hr for processing. Frogs were sacrificed by sodium pentobarbital (Nembutal®) overdose. Methods for necropsy and preparation and staining of parasites follow McAllister et al. (1989). For coccidial isolation, individual samples of rectal contents and feces in Hank's balanced salt solution (HBSS) were initially examined for coccidia using Brightfield microscopy following flotation in Sheather's sucrose solution (sp. gr. 1.30). Positive samples containing unsporulated oocysts were placed in individual Petri dishes containing a thin layer of HBSS supplemented with 100 IU penicillin G/ml and 100 µg streptomycin/ml. Following a sporulation period of 5 days at room temperature (ca. 23°C), oocysts were mailed to Kansas State University. Oocysts were concentrated by flotation, measured using a calibrated ocular micrometer, and examined and photographed using Nomarski interference-contrast optics. Measurements are reported in micrometers (µm) with means followed by the ranges in parentheses. Oocysts were 1.5 wk old when measured and photographed.

Symbiotypes of *R. sylvatica* from which parasites were collected are deposited in the Arkansas State University Museum of Zoology (ASUMZ 19434–19446). Voucher specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Opalina* sp. (USNM 83928), *Myxidium serotinum* (USNM 83927), *Brachycoelium salamandrae* (USNM 83925), unidentifiable trematode metacercariae (USNM 83926), *Mesocestoides* sp. tetrahyridia (USNM 83929), *Abbreviata* sp. (USNM 83931), and *Oswaldocruzia pipiens* (USNM 83930).

Results and Discussion

Three protozoan and 6 metazoan parasites infected *R. sylvatica* (Table 1). Of the 13 wood frogs

examined, 1 (8%) was uninfected and 12 (92%) harbored multiple infections. None of the frogs were infected with apicomplexan or trypanosomal parasites in the blood.

Endocommensal *Opalina* sp. Purkinje and Valentin, 1840, not identifiable to species, were found in the rectum of 8 *R. sylvatica* (56.5 ± 1.9, 49–66 mm SVL). *Opalina virguloidea* Metcalf, 1923, has been reported from *R. sylvatica* in Ohio and North Carolina (Metcalf, 1923) and *O. obtrigonoidea* Metcalf, 1923, was reported from wood frogs in Ohio (Odlaug, 1954). In addition, Metcalf (1923) reported *Cepedea cantabrigensis* from *R. cantabrigensis* (= *R. sylvatica*) from Manitoba, Canada, Alaska, and Michigan. A similar opalinid was recently reported by McAllister et al. (1995b) in the pickerel frog, *Rana palustris*, from Independence County, Arkansas.

Trophozoites and spores of the myxosporean, *Myxidium serotinum* Kudo and Sprague, 1940, were found in 3 frogs (57.7 ± 5.5, 49–68 mm SVL). This represents a new host record and the second time *M. serotinum* has been reported from Arkansas, as McAllister et al. (1995b) recently recovered the parasite from *R. palustris*. *Myxidium serotinum* also infects other members of the Ranidae, including *Rana* sp. and *R. clamitans* from Louisiana, *R. pipiens* from unspecified locales in the United States, and *R. utricularia* from Florida (Kudo and Sprague, 1940; Kudo, 1943).

Specimens of the plagiocercid trematode, *Brachycoelium salamandrae* (Frölich, 1789) Du-

jardin, 1845, were found in 4 frogs (64.0 ± 3.7 , 53–69 mm SVL) with a mean intensity of 2.5 ± 0.9 (range 1–4) worms per host. This parasite has been reported previously in wood frogs from Michigan (Najarian, 1955) and Ohio (Odlaug, 1954). McAllister et al. (1995a, b) reported *B. salamandrae* from Arkansas in graybelly salamanders, *Eurycea multiplicata griseogaster* and *R. palustris* (respectively).

Unidentified trematode metacercariae were found encapsulated in tissues of 5 frogs (62.0 ± 3.5 , 52–69 mm SVL). Various unidentified, echinostome, and gorgoderid metacercariae have been reported in *R. sylvatica* from Massachusetts and Michigan (Rankin, 1945; Najarian, 1955; Muzzall and Peebles, 1991).

Numerous tetrathyridia of the cyclophyllidean tapeworm, *Mesocestoides* sp. Vaillant, 1863, were encapsulated in tissues within the body cavity of 2 *R. sylvatica* (53 and 58 mm SVL). This represents a new host record for *Mesocestoides* sp. McAllister et al. (1995b) recently reported *Mesocestoides* sp. tetrathyridia in *R. palustris* from Arkansas. In addition, other ranid hosts include *R. berlandieri* from Texas, *R. clamitans* from Wisconsin, and *R. pipiens* from Iowa, Minnesota, New York, South Dakota, and Wisconsin (see McAllister and Conn, 1990).

A single third-stage larval spiruroid nematode, *Abbreviata* sp. Travassos, 1920, was found in a 56-mm SVL wood frog. Walton (1931) provided a description of *Physaloptera* (= *Abbreviata*) *ranae* from *R. sylvatica* based on larval specimens. However, Baker (1987) designated *A. ranae* a species inquirenda. McAllister et al. (1993, 1995b) also reported *Hyla avivoca* and *R. palustris* from Arkansas as hosts of *Abbreviata* sp. Other ranids infected with *Abbreviata* sp. include *R. catesbeiana*, *R. clamitans*, *R. pipiens*, and *R. sphenocéphala* (Walton, 1931; Morgan, 1945; Baker, 1987).

A single male stronglylid nematode *Oswaldocruzia pipiens* Walton, 1929, was found in a 55-mm SVL *R. sylvatica*. This nematode has been reported previously in *R. sylvatica* from Canada (Baker, 1978a), Massachusetts (Rankin, 1945), Michigan (Muzzall and Peebles, 1991), and New York (Harwood, 1930, 1932). McAllister et al. (1993, 1995b) reported the species in *H. avivoca* and *R. palustris* from Arkansas. There is little host specificity in this parasite, as other frogs (ranids and hylids), toads, salamanders, turtles, and lizards have been reported as hosts of *O. pipiens* (see Baker, 1987).

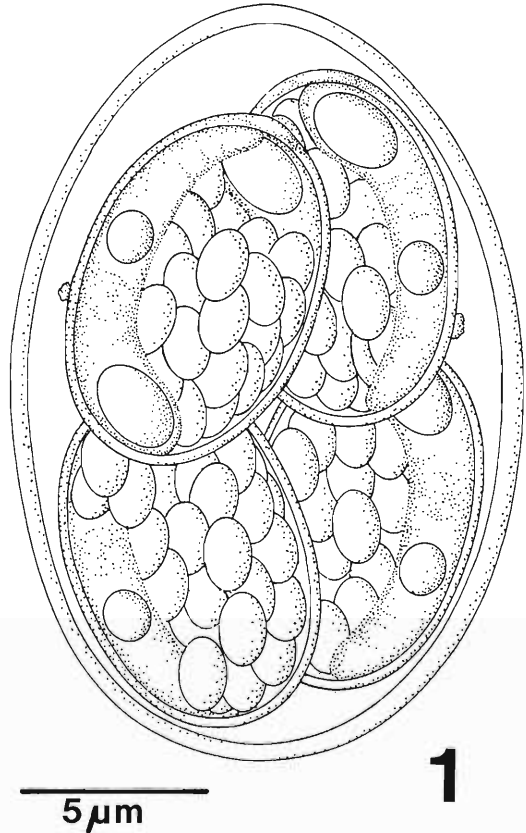
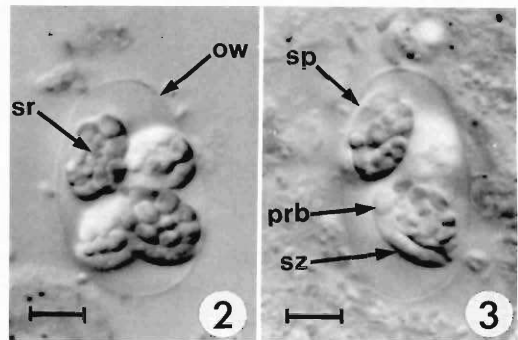


Figure 1. Composite line drawing of sporulated oocyst of *Eimeria fitchi* sp. n. from *Rana sylvatica*.

A glossiphoniid leech, *Desserobdella* (syn. *Batrachobdella*) *picta* (Verrill, 1872) was found firmly attached to the dorsal skin of a male *R. sylvatica* (56 mm SVL). This leech is widely dis-



Figures 2, 3. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria fitchi* sp. n. Abbreviations: ow = oocyst wall, prb = posterior refractile body, sp = sporocyst, sr = sporocyst residuum, sz = sporozoite. Scale bars = 5.0 μ m.

Table 2. Helminths reported from *Rana sylvatica* from various North American localities.

Helminth	Locality	Reference
Trematoda		
<i>Alaria arisaemoides</i> *	Canada	Pearson, 1956
<i>Brachycoelium salamandrae</i>	Arkansas	This report
	Michigan	Najarian, 1955
	Ohio	Odlaug, 1954
Echinostome cysts	Michigan	Najarian, 1955
<i>Glythelmins quieta</i>	Michigan	Muzzall and Peebles, 1991
Gorgoderid cysts	Massachusetts	Rankin, 1945
	Michigan	Najarian, 1955
<i>Gorgoderina attenuata</i>	Massachusetts	Rankin, 1945
<i>G. translucida</i>	Idaho	Waitz, 1961†
	Maine	Bouchard, 1951
<i>Haematoloechus complexus</i>	Ohio	Catalano and White, 1977
<i>H. medioplexus</i>	Wisconsin	Williams and Taft, 1980
<i>H. parviplexus</i>	Michigan	Muzzall and Peebles, 1991
<i>H. varioplexus</i>	Idaho	Waitz, 1961†
	Michigan	Najarian, 1955
<i>Megalodiscus temperatus</i>	Canada	Stafford, 1905
Unidentified metacercariae	Arkansas	This study
	Michigan	Muzzall and Peebles, 1991
Cestoidea		
<i>Cylindrotaenia americana</i>	Canada	Jones, 1987
<i>Mesocestoides</i> sp.	Arkansas	This report
Plerocercoid larva (cysts)	Massachusetts	Rankin, 1945
Nematoda		
<i>Abbreviata</i> sp.	Arkansas	This report
	Maryland	Walton, 1931
<i>Cosmocercoides dukae</i>	Michigan	Muzzall and Peebles, 1991
or <i>C. variabilis</i>	New York	Harwood, 1930, 1932
	Ohio	Odlaug, 1954
<i>Gyrinicola batrachiensis</i> ‡	Canada	Adamson, 1980
<i>Megalobatrachonema gigantica</i>	Idaho	Waitz, 1961†
<i>Microfilaria ranae-sylvaticae</i> §	Canada	Fantham et al., 1942
<i>Oswaldocruzia pipiens</i>	Arkansas	This report
	Massachusetts	Rankin, 1945
	Michigan	Muzzall and Peebles, 1991
	New York	Harwood, 1932
	Canada	Baker, 1978a
<i>Rhabdias ranae</i>	Canada	Baker, 1978b, 1979a, b
	Massachusetts	Rankin, 1945
	Michigan	Muzzall and Peebles, 1991
	Wisconsin	Williams and Taft, 1980
<i>Spiroxys</i> sp.	Michigan	Muzzall and Peebles, 1991
Acanthocephala		
Unidentified cystacanth	Ohio	Odlaug, 1954

* Experimental infection in tadpoles.

† Reported from *Rana pretiosa* (spotted frog) × *R. sylvatica* hybrids.

‡ Only tadpoles are reported to be infected.

§ Considered a species inquirenda by Baker (1987).

tributed in the United States and was reported previously from southeastern Arkansas (see Klemm, 1982). It is found in small woodland ponds and typically arrives shortly before breeding aggregations of amphibians. There is little

host specificity in *D. picta*, as other hosts include *Ambystoma maculatum*, *A. talpoideum*, *A. tigrinum*, *Bufo americanus*, *Hyla versicolor*, *Pseudacris crucifer*, *R. clamitans*, *R. catesbeiana*, and *R. septentrionalis* (Sawyer, 1972; Sawyer and

Shelly, 1976; Klemm, 1982; Barta and Dessler, 1984).

In addition to the parasites already noted, numerous eimerian oocysts were found in the feces of *R. sylvatica* (58.4 ± 2.0 , 49–69 mm), which proved to be the most commonly observed parasite in this host sample. On further examination, these oocysts were found to represent a previously undescribed species. Here we present a description of this new coccidian.

Eimeria fitchi sp. n.
(Figs. 1–3).

DESCRIPTION OF OOCYSTS: Oocysts ovoidal, contents with light greenish tint, 21.9×14.3 ($20.0\text{--}24.0 \times 13.2\text{--}15.2$) ($n = 25$), with smooth, thin, single-layered wall ca. 0.5 thick; shape index (length/width) 1.5 (1.3–1.7). Micropyle, oocyst residuum, and intact polar granule absent, although 1–3 fragments are sometimes seen attached to outer walls of sporocysts. Sporocysts ovoidal, 10.9×7.4 ($9.8\text{--}11.2 \times 7.0\text{--}8.0$), with smooth, thin, single-layered wall; shape index 1.5 (1.3–1.6). One end of sporocyst thickened slightly to form what appears to be indistinct Stieda body; substieda body absent. Sporocyst residuum present, 3.6×1.6 , consisting of about 25 large, coarse granules often scattered free among sporozoites. Sporozoites elongate, 11.1×1.7 ($10.4\text{--}12.0 \times 1.6\text{--}1.8$) in situ, each with 2 refractile bodies. Spherical antero-central refractile body, 1.1 (0.8–1.4) in diameter; posterior refractile body subspherical to ovoidal, 2.9 long \times 1.6 wide ($2.2\text{--}3.4 \times 1.4\text{--}1.6$). An indistinct nucleus located between refractile bodies.

TYPE HOST: *Rana sylvatica* LeConte, 1825, “wood frog” (Anura: Ranidae), adult male, 69 mm SVL, collected 20 February 1994 by S. E. Trauth. Symbiotype deposited as ASUMZ 19434.

TYPE SPECIMENS: Phototype (see Bandoni and Duszynski, 1988) of sporulated oocysts in the U.S. National Parasite Collection, Beltsville, Maryland, as USNMPC No. 84163.

TYPE LOCALITY: 6.0 km SW Melbourne, off State Hwy 9, Izard County, Arkansas.

PREVALENCE: Found in 11 (85%) of the 13 frogs examined.

SITE OF INFECTION: Unknown. Oocysts recovered from rectal contents and feces.

SPORULATION: Exogenous. All oocysts were passed unsporulated or partially sporulated and became fully sporulated within 5 days at ca. 23°C.

ETYMOLOGY: The specific epithet is given in honor of Henry S. Fitch, Professor Emeritus,

University of Kansas, in recognition of his numerous contributions to our understanding of the natural history and ecology of North American amphibians and reptiles.

REMARKS: Oocysts of *E. fitchi* sp. n. can be distinguished from *E. kermitti* Chen and Dessler, 1989, and *E. algonquini* Chen and Dessler, 1989, from *R. sylvatica* in Ontario, Canada, as follows: oocysts of *E. kermitti* are larger and possess an oocyst residuum and polar granule, and sporocysts have a distinct Stieda body; oocysts of *E. algonquini* are spherical, and sporocysts are distinctly different (see Chen and Dessler, 1989). Further, oocysts of *E. fitchi* sp. n. are unlike those found in other anurans, including *Rana* spp. (see Upton and McAllister, 1988). *Rana sylvatica* represents the first ranid frog from the United States known to harbor coccidia.

In summary, parasites of our sample of Arkansas *R. sylvatica* were similar to those reported from other surveys on *R. sylvatica* from various parts of its range (Table 2). We also noted that several parasites of *R. sylvatica* are shared with *R. palustris* and *H. avivoca* in Arkansas. This was not surprising given that many anuran parasites have a wide geographic range and exhibit little host specificity. Admittedly, our sample size was small, and the survey lacked data for female *R. sylvatica*. In the most exhaustive helminth survey on *R. sylvatica* to date, Muzzall and Peebles (1991) reported 3 trematode and 4 nematode parasites in 100 wood frogs (combined prevalence = 77%) from Michigan. However, all frogs were reported to be under 47 mm SVL and, as such, represent juveniles. Therefore, additional surveys on protozoan and metazoan parasites of *R. sylvatica* should include examination of all age and size classes of male and female wood frogs collected throughout the year.

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Meeting Schedule

1995–1996

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| 11 October 1995 | National Institutes of Health (NIH), Bethesda, MD.
Contact: Louis Miller (301) 496-2183. |
| 8 November 1995 | Anniversary Dinner, Uniformed Services University of Health Sciences (USUHS), Bethesda, MD.
Contact: John Cross (301) 295-3139. |
| 14 February 1996 | Nematology Laboratory, United States Department of Agriculture, Beltsville, MD.
Contact: David Chitwood (301) 504-5660. |
| 20 March 1996 | Johns Hopkins Montgomery County Center, Rockville, MD.
Contacts: Thomas Simpson (410) 366-8814 and Alan Scott (410) 955-3442. |
| 4 May 1996 | New Bolton Center, University of Pennsylvania, Kennett Square, PA.
Contact: Gerhard Schad (215) 898-6680. |