
Commensal Protista, Cnidaria and Helminth Parasites of the Cajun Chorus Frog, *Pseudacris fouquettei* (Anura: Hylidae), from Oklahoma

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Abstract: Twenty adult Cajun chorus frogs (*Pseudacris fouquettei*) were collected in McCurtain County, Oklahoma, and examined for commensal protozoans and helminth parasites. All 20 frogs harbored with one or more species, including 13 each (65%) with *Opalina* sp., and *Nyctotherus cordiformis*, 10 (50%) with *Cystodiscus melleni*, three (15%) with unknown reniferid metacercaria, three (15%) with *Mesocoelium* sp., one (5%) with *Cylindrotaenia americana*, four (20%) with *Oswaldocruzia leidy*, five (25%) with *Cosmocercoides variabilis*, and three (15%) with unidentified acuariid larva. All (100%) harbored two or more protists, a cnidarian and/or helminths each. The *Mesocoelium* sp. appears to be a new species and new host records are reported for it as well as reniferid metacercaria; new distributional records in the state are documented for *N. cordiformis*, *C. melleni*, *Mesocoelium* sp. and *O. leidy*. ©2015 Oklahoma Academy of Science

Introduction

The Cajun chorus frog (*Pseudacris fouquettei*) ranges from extreme southern Missouri south to western Mississippi, through all of Louisiana and Arkansas, and west to eastern Texas and Oklahoma (Lemmon et al. 2008). In Oklahoma, *P. fouquettei* is found in the central and eastern part of the state in partly wooded areas and prairies (Sievert and Sievert 2011). It is one of the first frogs to call in the winter and breeds during and after heavy rains in early spring; an adult frog is rarely found except during the breeding season.

McAllister et al. (2008) reported the cnidarian (myxozoan), *Cystodiscus* (= *Myxidium*) *melleni* from *P. fouquettei* (as *P. triseriata feriarum*) from Texas, and more recently, McAllister et al. (2013a) reported on protozoan and helminth parasites of *P. fouquettei* from Arkansas and Texas. Several helminths, including three digeneans (*Brachycoelium salamandrae*, *Glypthelmins quieta*, *Mesocoelium monas*), a tapeworm (*Cylindrotaenia americana*), and five nematodes (*Cosmocercoides variabilis*, *Oswaldocruzia leidy*, *O. pipiens*, *Physaloptera* sp., and acuariid larvae) have been previously reported from *P. fouquettei* from Arkansas (McAllister et al. 2013), Oklahoma (as *P. nigrita*

triseriata, *P. triseriata* or *P. feriarum*, Kuntz 1941) and Texas (as *P. triseriata*, Harwood 1930, 1932; McAllister et al. 2013a). Here, for the first time for a moderately-sized population from Oklahoma, we report new information on commensal protists, a cnidarian and helminth parasites of *P. fouquettei* in a survey of individuals from the southeastern part of the state.

Methods

During 11–13 March 2015, 20 adult (18 male, 2 female) *P. fouquettei* (mean \pm 1SD snout–vent length [SVL] = 32.2 ± 2.7 , range 27–39 mm) were collected by hand from temporary wetland in Hochatown off US 259 in McCurtain County (34.162096°N, 94.755017°W). Specimens were placed on ice in individual bags and taken to the laboratory within 24 hr for necropsy. Frogs were overdosed by immersion in a concentrated chlorethone solution and a mid-ventral incision from mouth to cloaca was made to expose the gastrointestinal (GI) tract. The entire GI tract from the mouth to cloaca was split lengthwise and along with gall bladder, kidneys, liver, lungs, and gonads were placed in Petri dishes and examined using a stereomicroscope. The eustachian tubes were not examined. Frogs were also examined for select protists, including the gall bladder for cnidarians, the rectum for opalinids and ciliates, and the feces for coccidia following methods of Upton and McAllister (1988), McAllister et al. (1989), and McAllister and Trauth (1995). Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, transferred to 70–95% DNA grade ethanol, stained with acetocarmine and mounted in Canada balsam. Nematodes were fixed in hot 70% ethanol and placed on a glass slide in a drop of undiluted glycerol for identification. Photovoucher and regular voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

Results and Discussion

All 20 of the *P. fouquettei* (Table 1) were found to harbor at least one of two commensal protists, a cnidarian and/or six helminths, including 13 (65%) with *Opalina* sp., and *Nyctotherus cordiformis* (Fig. 1A), 10 (50%) with *Cystodiscus melleni* (Figs. 1B–C), three (15%) with numerous reniferid metacercaria (Figs. 1D–F), three (15%) with *Mesocoelium* sp. (Fig. 1G), one (5%) with a single *Cylindrotaenia americana*, four (20%) with five female *Oswaldocruzia leidyi*, five (25%) with 10 (2 male, 3 female, 5 immature) *Cosmocercoides variabilis*, three (15%) with numerous acuariid larvae (Fig. 1H). All (100%) harbored multiple infections of two or more protists, cnidarians and/or helminths each. None of the *P. fouquettei* was passing coccidian oocysts in feces at the time they were sampled.

The commensal protist, *Nyctotherus cordiformis* (Ehrenberg, 1838) Stein, 1867 as well as commensal *Opalina* sp. have been reported previously from *P. fouquettei* from Arkansas and Texas (McAllister et al. 2013a). Trowbridge and Hefley (1934) reported an *Opalina* sp. in several Oklahoma anurans. A “very light” infection of an unidentified *Nyctotherus* sp. was reported in a Texas horned lizard (*Phrynosoma cornutum*) from Norman, Oklahoma, by Zimmerman and Brown (1952). However, we are unaware of any published report of *N. cordiformis* in Oklahoma anurans. Although this protist is cosmopolitan in distribution, we document it (HWML photovoucher 101835) in an Oklahoma frog for the first time.

Cystodiscus (syn. *Myxidium*) *melleni* Jirků, Bolek, Whipps, Janovy, Kent, and Modrý, 2006 was reported originally from western chorus frog (*Pseudacris triseriata*) and Blanchard’s cricket frog (*Acris blanchardi*) from Nebraska (Jirků et al. 2006). Since then, this cnidarian was found in *P. fouquettei* in Arkansas (McAllister et al. 2013a) and Texas (McAllister et al. 2008). Ribosomal DNA sequencing of trophozoites and free spores (HWML photovoucher 101836) from our Oklahoma *P. fouquettei* confirmed the identity as *C. melleni* (C. Whipps, pers. comm.). We add

Table 1. Presence (+) or absence (-) of each parasite/commensal from 20 individual *Pseudacris fouquettei* in Oklahoma.

Parasite/Commensal	Frog Number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Protista																				
<i>Nyctotherus cordiformis</i> *	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
<i>Opalina</i> sp.	+	-	+	+	-	-	+	+	+	-	-	+	-	+	+	-	-	+	+	+
Cnidaria																				
<i>Cleidodiscus mellini</i> *	-	+	-	+	+	+	-	+	-	-	-	+	-	-	-	+	-	+	+	+
Trematoda																				
<i>Mesocoelium</i> sp. *, †	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+
<i>Renijer metacercaria</i> †	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Cestoda																				
<i>Cylindrotaenia americana</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nematoda																				
Acutariid larvae	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Cosmoecoides variabilis</i>	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oswaldocruzia leidyi</i> *	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	-	-

*New distributional record.

†New host record.

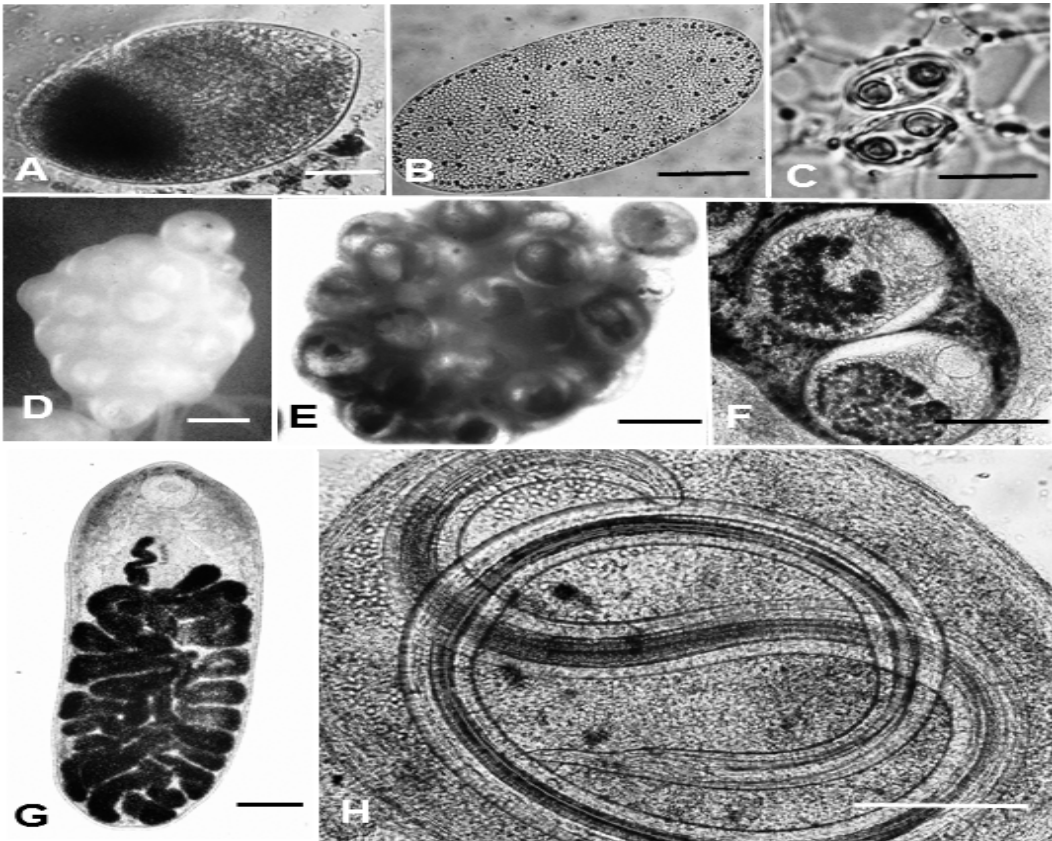


Figure 1. Commensal protists, a cnidarian, and helminths from *Pseudacris fouquettei* from Oklahoma (all unstained). **A.** *Nyctotherus cordiformis*. Scale bar = 50 μm . **B.** *Cystodiscus melleni* trophozoite (plasmodium). Scale bar = 100 μm . **C.** *Cystodiscus melleni* spores. Scale bar = 10 μm . **D.** Macroscopic view of reniferid metacercariae in cysts. Scale bar = 1 mm. **E.** Another view of same cysts. Scale bar = 1 mm. **F.** Two reniferid metacercariae in cysts. Scale bar = 500 μm . **G.** *Mesocoelium* sp. Scale bar = 200 μm . **H.** Acuariid larvae in cyst. Scale bar = 200 μm .

Oklahoma to the geographic range of *C. melleni*.

Unidentified reniferid metacercariae (HWML 91942, photovoucher 101837) were found encapsulated in the coelomic cavity of *P. fouquettei* (Figs. 1D-F). Numerous (> 100) metacercariae appeared grouped together similar to the appearance of a “cluster of berries” (see Fig. 1D). *Renifer* (syn. *Ochetosoma*) and *Pneumatophilus* spp., as adults, occur in the oral cavity, esophagus and lungs of mostly natricine and other snakes (Tkach 2008). This is the first time, to our knowledge, these kind of metacercariae have been reported from this anuran host.

McAllister et al. (2013a) previously reported *Mesocoelium monas* (Rudolphi, 1819) Frietas, Proc. Okla. Acad. Sci. 95: pp 86 - 92 (2015)

1958 from one of 14 (7%) *P. fouquettei* from Arkansas. Recently, Calhoun and Dronen (2012) reevaluated specimens previously identified as *M. monas* and proposed keys to some 43 species. Our species of *Mesocoelium*, however, differs from *M. monas* by having a genital pore bifurcal compared with prebifurcal (Calhoun and Dronen 2012; Dronen et al. 2012). Dronen et al. (2012) divided the genus of *Mesocoelium* into six body types based primarily on cecal length, genital pore location compared to cecal bifurcation, and finally whether the genital pore is median or submedian on the body. Using these key characters developed by Dronen et al. (2012) our specimens of *Mesocoelium* (HWML photovoucher 101838) places them in the *Mesocoelium sociale* (Lühe, 1901) Odhner,

Table 2. Summary of commensals and parasites reported from *Pseudis fouquettei* from Arkansas, Oklahoma and Texas.

Commensal/Parasite	State	Prevalence*	Intensity†	Reference
Protista				
<i>Nyctotherus cordiformis</i>	AR	13/14 (93%)	–	McAllister et al. (2013)
	OK	13/20 (65%)	–	This study
	TX	2/5 (40%)	–	McAllister et al. (2013)
<i>Opalina</i> sp.	AR	13/14 (93%)	–	McAllister et al. (2013)
	OK	13/20 (65%)	–	This study
	TX	5/5 (100%)	–	McAllister et al. (2013)
Cnidaria				
<i>Cleidodiscus melini</i>	AR	6/14 (43%)	–	McAllister et al. (2013)
	OK	10/20 (50%)	–	This study
	TX	1/6 (17%)	–	McAllister et al. (2008)
Trematoda				
<i>Brachycoelium salamandrae</i> ‡	TX	2/26 (8%)	not given	Harwood (1932)
	OK	1/3 (33%)	not given	Kuntz (1940) §
	TX	2/26 (8%)	not given	Harwood (1932)
<i>Megalodiscus temperatus</i>	OK	3/20 (15%)	2.6 ± 2.1 (1–5)	This study
<i>M. monas</i>	AR	1/14 (7%)	1	McAllister et al. (2013)
<i>Renifer metacercaria</i>	OK	3/20 (15%)	–	This study

Unknown metacercaria	OK	3/25 (12%)	–	Bouchard (1953)#
Cestoda				
<i>Cylindrotaenia americana</i>	OK	1/25 (4%) 1/20 (5%)	1 1	Bouchard (1953)# This study
Nematoda				
Acuariid larvae	OK	3/20 (15%)	–	This study
	TX	1/5 (20%)	1	McAllister et al. (2013)
<i>Cosmoceroides variabilis</i>	AR	2/14 (14%)	1, 1	McAllister et al. (2013)
	OK	5/20 (25%)	2.5 ± 1.0, 2–4	This study
	TX	16/26 (62%)	not given	Harwood (1930,1932)¶
<i>Physaloptera</i> sp.	TX	1/5 (20%)	1	McAllister et al. (2013)
<i>Oswaldocruzia leidyi</i>	AR	2/14 (14%)	1, 1	McAllister et al. (2013)
	OK	4/20 (20%)	1.3 ± 0.5, 1–2	This study
	TX	1/5 (20%)	3	McAllister et al. (2013)
<i>O. pipiens</i>	OK	5/25 (20%)	4?	Bouchard (1953)#

*Prevalence = number infected/number examined (%).

†mean ± 1SD (range), where applicable or (–) too numerous to count.

‡Parasite originally reported as *Brachycoelium daviesi* (= *B. salamandrae*).

¶Host reported as *Pseudacris triseriata* (= *P. fouquettei*) per Lemmon et al. (2008).

§Unpublished thesis; Kuntz (1941) did not specify what host species were infected.

#Unpublished dissertation.

1910 body type by having ceca that surpass the ovary and a genital pore that is bifurcal and submedian. *Mesocoelium sociale* has previously been described from Asian black-spotted toad (*Duttaphrynus melanostictus*) in northern India and giant Asian toad (*Phrynooidus asper*) in Malaysia (Dronen et al. 2012). To date, *M. sociale* has not been reported from the United States. Our specimens differ from *M. sociale* by having a smaller oral sucker width (175–195 vs. 200–225 μm), slightly smaller oral sucker to pharynx ratio (1:2.0 vs. 1:2.1–1:2.3), larger oral sucker to ventral sucker ratio (1:1.4–1:1.6 vs. 1:1.2–1:1.3), and longer eggs (42.5–45.0 vs. 38–40 μm). Other amphibian hosts of *M. sociale* include *Fejervarya* (= *Rana*) *cancrivora*, *Hylarana erythraea*, *Kaloula baleata*, *K. pulchra*, and *Polypedates* (= *Rhacophorus*) *leucomystax* from Malaysia and Thailand (Fischthal and Kuntz 1965; Wongsawad et al. 1998).

Mesocoelium sociale was also reported from the GI tract of several lizards, including *Anolis sagrei*, *Bronchocela* (= *Calotes*) *crystalinus*, *Eutropis longicaudata*, *Japalura swinhonsis*, *Plestiodon elegans*, and *Sphenomorphus indicus* from Taiwan (Fischthal and Kuntz 1975; Norval et al. 2011, 2014; Goldberg et al. 2014), and *Hemidactylus frenatus*, *Cosymbotus platyurus*, *C. versicolor*, *Gecko gecko*, *Mabuya multifasciata* from Indonesia and Malaysia (Killick and Beverley-Burton 1982; Kennedy et al. 1987).

In the end, there are significant zoogeographical and anuran host family differences as our specimens of *Mesocoelium* were discovered in hylid frogs from the southwestern United States compared with *M. sociale* from bufonid toads from India and Malaysia. Therefore, we believe our specimens of *Mesocoelium* represent a new species and future study will include molecular analyses (V. Tkach, pers. comm.).

The tapeworm, *Cylindrotaenia americana* Jewell, 1916 is a commonly-encountered parasite of the small intestine of various amphibians, particularly anurans (McAllister et al. 2013b). It has been previously reported from Great Plains toad (*Anaxyrus cognatus*), Blanchard's cricket

frog (*Acris blanchardi*), American bullfrog (*Lithobates catesbeianus*), southern leopard frog (*Lithobates sphenoccephalus utricularius*) and dwarf American toad (*Anaxyrus americanus charlesmithi*) in Oklahoma (Trowbridge and Hefley 1934; McAllister et al. 2014; Vhora and Bolek 2015). Harwood (1932) reported *C. americana* in *P. fouquettei* (as *P. triseriata*) from Houston, Texas. However, we document *C. americana* (HWML 91943) for the first time in Oklahoma *P. fouquettei*.

Cosmocercoides variabilis (Harwood, 1930) Travassos, 1931 has previously been reported from the state in *A. a. americanus*, *L. catesbeianus*, Sequoyah slimy salamanders, *Plethodon sequoyah* and Hurter's spadefoot, *Scaphiopus hurterii* (Trowbridge and Hefley 1934; McAllister and Bursey 2004; McAllister et al. 2005, 2014). This nematode also has been previously reported from *P. fouquettei* in Arkansas (McAllister et al. 2013a). It is reported here (HWML 91944) from an Oklahoma population of *P. fouquettei* for the first time.

The strongylid nematode, *Oswaldocruzia leidy* Steiner, 1924 was reported from *P. fouquettei* from Arkansas and Texas (McAllister et al. 2013a). Trowbridge and Hefley (1934) were the first to report a similar species, *Oswaldocruzia pipiens* Walton, 1929 from Oklahoma in *Lithobates* spp. and Woodhouse's toad, *Anaxyrus woodhousii*. Kuntz and Self (1944) reported an *Oswaldocruzia* sp. from an unspecified anuran host. In an unpublished dissertation, Bouchard (1953) reported *O. pipiens* from *P. fouquettei* (as *P. triseriata*) from Oklahoma. Although *O. pipiens* was earlier reported from *P. fouquettei* (as *P. nigrita triseriata*, *P. triseriata* or *P. feriarum*) in Oklahoma by Kuntz (1941) and numerous other anurans from Arkansas, Florida, Georgia, Louisiana, Maine, Ohio, Texas, and Virginia, and Alberta and Ontario, Canada (see references in McAllister et al. 2013a), we document *O. leidy* (HWML 91945) in an Oklahoma host for the first time.

Unidentified acuariid larvae (Spirurida) were previously reported in *P. fouquettei* from

Texas (McAllister et al. 2013a). Species within the Acuariidae are primarily parasites of birds, although several species have been reported from mammals and frogs, like *P. fouquettei*, may serve as paratenic hosts (Anderson 2000). This is the second time these nematodes (HWML 91946, photovoucher 101839) have been reported from Oklahoma (see McAllister et al. 2014).

The Cajun chorus frog has now been the subject of helminth surveys from specimens collected in Arkansas, Oklahoma and Texas. To date, the helminth list of *P. fouquettei* includes seven trematode species, one tapeworm species, and five nematode species (Table 2). When compared to previous surveys on this host, all of the helminths, except *Mesocoelium* sp. and the reniferid metacercaria have been reported from *P. fouquettei*. However, new distributional records are documented in the state for *N. cordiformis*, *C. melleni*, *Mesocoelium* sp. and *O. leidyi*. As the host range also includes Missouri, and, more importantly, sites east of the Mississippi River in western Mississippi, surveys should include *P. fouquettei* from those states in order to fully compare their endoparasites with previous surveys from other states. Furthermore, future research needs to include molecular sequencing to differentiate between interspecific and intraspecific morphological variation in the genus *Mesocoelium*.

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