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Ecology of the Squirrel Treefrog (*Hyla squirella*) in Southern Arkansas

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Running Title: Ecology of the Squirrel Treefrog

Abstract

We conducted an ecological study of the Squirrel Treefrog, *Hyla squirella* near El Dorado, Union Co., Arkansas from May-Oct. 2013. We extended the known distribution by ~2 km and documented the first breeding occurring on 28 May and the first transformation of juveniles on 27 Aug. Three endoparasites were documented: *Opalina* sp., *Nyctotherus cordiformis*, and *Physaloptera* sp. larvae. We also provide information on endoparasites of Florida *H. squirella* as well as a summary of helminths of this frog.

Introduction

Hyla squirella Bosc, the Squirrel Treefrog, is a small hyloid frog found throughout the southeastern United States (Conant and Collins 1998; Fig. 1). It was only recently discovered to occur in Arkansas, with the first record on 21 May 2013 (Fulmer and Connior 2013). This frog is distributed throughout Louisiana (Dundee and Rossman 1989), and, in fact, is known to occur ~80 km from the Arkansas location in nearby Ouachita Parish, Louisiana (Dundee and Rossman 1989, Fulmer and Connior 2013). Since this species was just detected in Arkansas in 2013, this study was conducted to elucidate the ecology of this species within Arkansas, specifically in regards to habitat, reproduction, and parasites. In addition, we provide information on some endoparasites of Florida *H. squirella* as well as a summation of the helminths of this frog.

Materials and Methods

During May-Jul. 2013, potential locales within ~5 km were searched near the discovery of the initial

population (Site 1; 33.2327°N; 92.6287°W) within Union Co. Individuals of *H. squirella* were collected by hand, measured for snout-vent length (SVL), and necropsied for parasite infection and reproductive status.

Additional ecological characteristics, such as number observed, numbers of males calling, reproductive activity, and other anuran species observed were noted as well. Specimens were placed in individual bags on ice and within 48 hr frogs were overdosed with a 10% v/v ethanol solution (HACC 2004). A mid-ventral incision from mouth to cloaca was made to expose the gastrointestinal tract. Specimens were examined for select protists, including the gall bladder for myxozoans and the rectum for opalinids and ciliates following McAllister (1987, 1991). Protists were processed for scanning electron microscopy following standard techniques used on other frogs (see McAllister et al. 2013) or were stained with Gomori trichrome for light microscopy. Nematodes were fixed in hot 70% v/v ethanol and placed on a glass slide in a drop of undiluted glycerol for identification. Voucher specimens of parasites were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland. Host voucher specimens were deposited in the Henderson State University Herpetological Collection (HSU 1712-1719), Arkadelphia.

Reproductive status of females was noted by the presence of ovarian eggs. When females were gravid, eggs were counted. We recorded egg counts or estimates of egg counts for five individuals. For the first female, we counted every egg within the abdominal egg mass and we estimated the egg counts for the remaining four. We determined the number of eggs in a volume that displaced 0.5 mL of water. Then, we estimated the total number of eggs by the volume of water displaced by the total egg mass. Final

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estimates were calculated by multiplying egg number in 0.5 mL of water by the total volume displaced.



Figure 1. *Hyla squirella* captured from Union County, Arkansas. Top (adult male); Bottom (recently metamorphosed juvenile).



Figure 2. *Hyla squirella* breeding site.

Results

One additional site was found (Site 2; 33.2142°N; 92.6315°W); a large breeding site ~2 km S of the original site near the Junction of US 63 and US 167 Bypass (Fig. 2). Individuals of *H. squirella* were collected by hand (four collected on 28 May 2013 [from Site 1] and three on 9 June 2013 [from Site 2]) and necropsied for parasite infection. In addition, 34 adult individuals were collected on 26 July 2013 for size and reproductive data, in addition to 12 recently metamorphosed juveniles collected from 28 August – 2 September 2013 from Site 2.

We documented numerous males calling (see Table 1) as well as pairs in amplexus (Fig. 3). We observed the following additional anurans calling from the same area: *Acris blanchardi*, *Anaxyrus fowleri*, *Hyla cinerea*, *H. chrysoscelis*, *Gastrophryne carolinensis*, and *Lithobates sphenoccephalus*. On 26 July 2013, we collected and measured 34 adults with a mean SVL of 31.8 mm (range = 29-35 mm) for 25 males and 33.8 mm (range = 30-37 mm) for 9 females. Of the 9 females, 7 were gravid. Total mean egg count estimates for the four individuals was 1324 eggs (range = 701-1635 eggs) per female. On 31 October 2013, after a heavy rain, we did not hear any *H. squirella* calling or observe any individuals.

We also observed that the younger tadpoles of *H. squirella* had golden dorsolateral stripes and older tadpoles were brown with golden flecks. Tail fins were clear, except for some dark mottling. Older tadpoles had white pigmentation on their throats. The mean SVL for 12 recently metamorphosed juveniles was 12.5 mm (range = 11-55 mm), with the first individual being observed on 16 August 2013 from Site 2.



Figure 3. *Hyla squirella* exhibiting amplexus.

Three species of endoparasites were found in *H. squirella*: *Opalina* sp., (USNPC 107672.02; Fig. 4a), *Nyctotherus cordiformis* (USNPC 107672.01; Fig. 4b), and three third-stage larval *Physaloptera* sp. (USNPC 107935); all in one of seven (14%) *H. squirella*. Each represents a new host record. We provide a summation of all previously reported helminths in *H. squirella* as well as previously unpublished records collected in Florida by the senior author in Table 2.

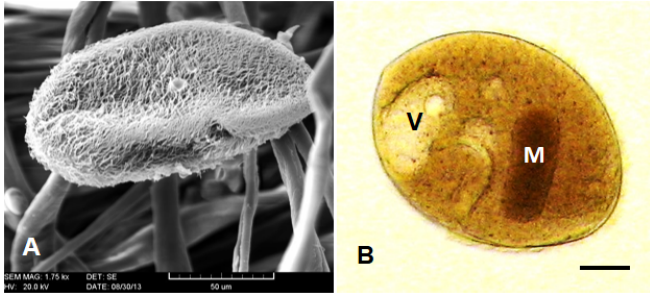


Figure 4. Protists from *Hyla squirella*. A. Scanning electron micrograph of *Opalina* sp. B. *Nyctotherus cordiformis* (unstained). Note macronucleus (M) and vacuole (V). Scale bar 25µm.

Discussion

Hyla squirella primarily reproduce during late spring and summer with calling choruses being recorded from Louisiana from March to November, and with calling individuals even being recorded in December (Dundee and Rossman 1989). We documented successful breeding of adults and metamorphosis of larvae for the first time in Arkansas (Fig. 1). Our reported breeding from May to July coincides with the typical breeding season reported in the literature. Our egg count of 989 is similar to two egg counts of 972 and 942 from Georgia (Wright 1932). Wright (1932) reported hatching within two days and an estimated larval period of 40-50 days, which coincides with the development at our site. Dundee and Rossman (1989) reported the only newly transformed individuals during their research from 25 September in Louisiana.

Both *Opalina* sp. and *N. cordiformis* were reported from every hyloid host that inhabits Arkansas (Muzzall and Sonntag 2012, McAllister et al. 2013). Both of these parasites are ubiquitous in amphibians. *Physaloptera* sp. nematode larvae was recently reported in Arkansas for the first time from the Cajun chorus frog, *Pseudacris fouquettei*, in Union County (McAllister et al. 2013). *Physaloptera* has been

reported previously from the hyloid frogs *Hyla versicolor* in Virginia and *Pseudacris crucifer* in North Carolina (Goldberg et al. 2009); both of these hyloid species also occur in Arkansas. All of these endoparasites are cosmopolitan in their ranges. This small hyloid is now host of three trematodes, one cestode, and five nematodes (Table 1). Although its parasite fauna is depauperate (Aho 1990), additional collections from this site and elsewhere will likely increase the number of helminths known from this host.

This study extended the known distribution of *H. squirella* within Arkansas; however it was only extended by about 2 km to the south. We suspect further systematic distributional surveys may produce additional breeding populations. Future surveys should include areas from southwestern to southeastern Arkansas, focusing on the southernmost tier of counties bordering Louisiana. As evidenced by the large breeding site, *H. squirella* can be quite numerous during breeding events in rainy weather, while going undetected during the rest of the year.

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Table 1. Ecological Notes of *Hyla squirella* from Union Co., Arkansas.

Locality	Date	Time (hrs)	Temp (°C)	Weather (rain)	Notes
Site 1	1 Jun 2013	2320	21.1	cloudy (8.9cm)	3 males calling 2 amplexant pairs
	2 Jun 2013	2130	22.2	clear	5 males calling
	18 Jun 2013	2120	25.6	cloudy (1.3cm)	one male calling
	23 Jul 2013	2300	25.6	cloudy (1.9cm)	6 males calling
	26 Jul 2013	2330	21.1	cloudy (6.3cm)	13 males calling
Site 2	7 Jun 2013	2230	20.0	clear	12 males calling
	8 Jun 2013	2115	23.3	clear	3 males calling
	9 Jun 2013	2100	23.9	clear	5 males calling
	26 Jul 2013	2330	21.1	cloudy (6.3cm)	>50 males calling >10 amplexant pairs
	27 Jul 2013	2300	20.6	clear	>50 males calling
	20 Sept 2013	2215	21.7	cloudy (8.9cm)	>25 males calling

Table 2. Summary of helminth parasites from *Hyla squirella*.

Helminth	Locality	Prevalence*	Reference
Trematoda			
<i>Lechriorchis tygartii</i> ^{1,2}	Florida	not given	Sears et al. (2012)
<i>Polystoma nearcticum</i>	Florida ⁴	4/14 (29%)	This report
	North Carolina	not given	Price (1939)
<i>Renifer aniarum</i> ^{1,2}	Florida	not given	Sears et al. (2012)
Cestoidea			
<i>Cylindrotaenia americana</i>	Florida ⁴	3/14 (21%)	This report
	Texas	1/11 (9%)	Harwood (1932)
Nematoda			
Acuariidae gen sp. ^{3,5}	Florida ⁴	1/14 (7%)	This report
<i>Cosmocercoides variabilis</i>	Florida ⁴	4/14 (29%)	This report
	Texas	2/11 (18%)	Harwood (1932)
<i>Gyrinicola batrachiensis</i> ²	Florida	1/1 (100%)	Pryor and Greiner (2004)
<i>Physaloptera</i> sp. ^{3,6}	Arkansas	1/7 (14%)	This report
	Florida ⁴	1/14 (7%)	This report
<i>Rhabdias ranae</i>	Florida	not given	Walton (1938)

*Number infected/number examined (percent).

¹Experimental infection.

²Tadpoles only.

³New host record.

⁴Previously unpublished records from Topsail Hill Preserve State Park, Walton Co., FL collected on 28 Mar. 2014 by MB Connior. Host vouchers (SVL = 26.6 ± 4.2 mm, range = 21-33 mm, *n* = 14) deposited in Arkansas State University Museum of Zoology (ASUMZ 33216-33226). Parasite vouchers deposited in USNPC.

⁵Larvae in cysts.

⁶Third-stage larvae.