

Endoparasites of Cope's Gray Treefrog, *Hyla chrysoscelis*, and Western Chorus Frog, *Pseudacris t. triseriata*, from Southeastern Wisconsin

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ABSTRACT: Sixty-five Cope's gray treefrogs, *Hyla chrysoscelis*, and 6 western chorus frogs, *Pseudacris t. triseriata*, were collected from southeastern Wisconsin during April through June 1996 and 1997 and examined for endoparasites. Fifty-one (78%) treefrogs and 5 (83%) chorus frogs were infected with 1 or more endoparasites. Two species of protozoa, 2 species of nematodes, 3 species of larval and adult digeneans, 1 species of monogenean, and 3 species of metacestodes infected the treefrogs. The chorus frogs were infected by 1 species of protozoan, 1 species of nematode, and 2 species of larval and adult digeneans. New host and distribution records for protozoan and metazoan parasites of Wisconsin anurans are reported.

KEY WORDS: *Hyla chrysoscelis*, *Pseudacris t. triseriata*, *Opalina* sp., *Nyctotherus cordiformis*, *Polystoma nearcticum*, *Glythelminis pennsylvaniensis*, *Mesocestoides* sp., *Cosmocercoides variabilis*, *Oswaldocruzia pi-piens*, Wisconsin.

Cope's gray treefrog, *Hyla chrysoscelis* Cope, 1880, is a large treefrog occurring in prairie ponds, oak savannas, dry and dry-mesic northern hardwoods, and lowland forests and has been distinguished from the eastern gray treefrog, *H. versicolor* Le Conte, 1825 (Ralin, 1968). Because of the similarity between these two species, different ranges have not been determined and little information on the natural history of *H. chrysoscelis* is available (Ralin, 1968; Jaslow and Vogt, 1977). The composite range covers most of the eastern United States from Ontario and southern Maine to the Gulf of Mexico (Vogt, 1981). The western chorus frog, *Pseudacris t. triseriata* Wied, 1839, is a small treefrog that ranges from the east end of Lake Ontario, west to central Minnesota, and south through Kansas and Oklahoma, with disjunct populations in New Mexico and Arizona (Vogt, 1981). A number of studies have been conducted on the endoparasites of the western chorus frog, but only two prior studies have been conducted on Cope's gray treefrog (see Table 1). Both species are common around moist prairies in southeastern Wisconsin, yet no studies exist on their endoparasites in this area. Here we present new information on the parasites of Wisconsin treefrogs.

Materials and Methods

Between May and June 1996 and April and June 1997, 65 Cope's gray treefrogs, 53 males and 12 females (mean \pm SD snout-vent length [SVL] = 41 \pm

3 mm, range = 33-48 mm) and 6 western chorus frogs, 4 males and 2 females (24 \pm 1 mm, 22-25 mm) were collected from two adjacent ephemeral ponds in Waukesha County, Wisconsin (42°54'N, 88°29'W). Cope's gray treefrogs were identified by mating call and mean erythrocyte length, as described by Bolek (1997). Specimens were collected by hand or dip-net during the night breeding chorus. Animals were transported to the laboratory and euthanized in MS 222 (ethyl m-aminobenzoate methane sulfonic acid) within 72 hr of capture. SVL and wet weight (WW) were recorded for each individual. At necropsy the digestive tract, limb and body wall musculature, and internal organs were examined for endoparasites. Intestinal protozoans were affixed to glass slides with albumin fixative and glycerol, fixed in modified Schaudinn's fixative, stained with hematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Nonencapsulated immature and adult digeneans and cestodes were relaxed and killed by slowly warming them in staining dishes containing 0.25% saline and then fixed in alcohol-formaldehyde-acetic acid (AFA). Monogeneans were relaxed under slight coverslip pressure in a refrigerator and then frozen and fixed in AFA, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Nematodes were killed in hot AFA, dehydrated to 70% ethanol, cleared in glycerol, and identified as temporary mounts. All tissue containing metacestodes was removed and fixed in 10% formalin, embedded in Paraplast, sectioned at 7 μ m, affixed to slides, stained with Harris's hematoxylin and eosin, and mounted in Canada balsam. All undigested stomach contents were identified to taxonomic class or order following Borror et al. (1989). Stomach contents were reported as a percentage, i.e., number of arthropods in a given class or order divided by total number of arthropods recovered. Prevalence, mean intensity, and abundance are according to Bush

Table 1. Endoparasites reported from *Hyla chrysoscelis* and *Pseudacris t. triseriata*.

Parasite species	<i>Hyla chrysoscelis</i>		<i>Pseudacris t. triseriata</i>	
	Locality	Reference	Locality	Reference
Protozoa				
<i>Opalina</i> sp.	Wisconsin Texas	This study Metcalf, 1923		
<i>O. obtrigonoidea</i>			Ohio	Odlaug, 1954
<i>O. chorophili</i>			Ohio	Odlaug, 1954
<i>Cepedea</i> sp.	Texas	Metcalf, 1923		
<i>Nyctotherus cordiformis</i>	Wisconsin	this study	Ohio Wisconsin	Odlaug, 1954 this study
<i>Trichomonas augusta</i>			Ohio	Odlaug, 1954
Digenea				
<i>Brachycoelium salamandrae</i>			Ohio	Odlaug, 1954
<i>Glythelmins hylareus</i>			Colorado Nebraska	Ubelaker et al., 1967 Brooks, 1976
<i>G. pennsylvaniensis</i>	Wisconsin	this study	Michigan Wisconsin	Muzzall and Peebles, 1991 this study
<i>G. quieta</i>			Ohio	Ashton and Rabalais, 1978
<i>Glythelmins</i> sp.			Indiana	Whitaker, 1971
<i>Haematoloechus complexus</i>	Nebraska	Brooks, 1976		
<i>Megalodiscus temperatus</i>	Nebraska	Brooks, 1976		
Unidentified immature trematode	Wisconsin	this study		
Unidentified metacercariae	Wisconsin	this study	Wisconsin	this study
Monogenea				
<i>Polystoma nearcticum</i>	Wisconsin	this study		
Cestoidea				
<i>Mesocestoides</i> sp.	Wisconsin	this study		
Unidentified plerocercoid	Wisconsin	this study		
Unidentified cestode cyst	Wisconsin	this study		
Nematoda				
<i>Cosmocercoides variabilis</i>	Wisconsin	this study	Ohio Wisconsin Texas Canada	Odlaug, 1954 this study Harwood, 1930 Vanderburgh and Anderson, 1987a
<i>C. dukae</i>			Ohio Michigan	Ashton and Rabalais, 1978 Muzzall and Peebles, 1991
<i>Oswaldocruzia pipiens</i>	Wisconsin	this study	Canada	Baker, 1977
<i>O. leidy</i>			Ohio	Ashton and Rabalais, 1978
<i>Falcaustra catesbeianae</i>			Arizona	Goldberg et al., 1996
<i>Gyrinicola batrachiensis</i> †			Canada	Adamson, 1981
<i>Rhabdias ranae</i>			Canada	Baker, 1978b

† In tadpoles.

et al. (1997). Voucher specimens have been deposited in the H. W. Manter Helminth Collection, University of Nebraska State Museum, Lincoln (accession numbers HWML 39468, *Opalina* sp.; 39483, *Nyctotherus cordiformis*; 39469, *Polystoma nearcticum*; 39470, *Glythelmins pennsylvaniensis*; 39471, immature trematode; 39472, unidentified metacercariae; 39473, unidentified plerocercoid; 39474, *Mesocestoides* sp.; 39482, unidentified cestode cyst; 39475, male *Cosmocercoides variabilis*; 39476, male *Oswaldocruzia pipiens*).

Results and Discussion

Eleven species of endoparasites infected Cope's gray treefrogs and 4 species infected western chorus frogs (Table 2). Of the 51 (78%) treefrogs infected with endoparasites, 26 harbored only 1 species, 19 harbored 2 species, and 6 harbored 3 species. Prevalence ranged from 52% for *Opalina* sp. to 1.5% for *Glythelmins pennsylvaniensis* Cheng, 1961. Overall mean

Table 2. Prevalence, abundance, and mean intensity of endoparasites of *Hyla chrysoscelis* and *Pseudacris t. triseriata* from southeastern Wisconsin.

	<i>Hyla chrysoscelis</i>			<i>Pseudacris t. triseriata</i>			
	Prevalence*	Mean (\pm SD) abundance	Mean (\pm SD) intensity (range)	Prevalence*	Mean (\pm SD) abundance	Mean (\pm SD) intensity (range)	Location†
Protozoa							
<i>Opalina</i> sp.	34 (52)	NC‡	NC	absent	absent	absent	SI, LI
<i>Nyctotherus cordiformis</i>	5 (8)	NC	NC	1 (17)	NC	NC	SI, LI
Monogenea							
<i>Polystoma nearcticum</i>	10 (15)	0.2 \pm 0.5	1.3 \pm 0.7 (1-3)	absent	absent	absent	UB
Digenea							
<i>Glypthelmis pennsylvanensis</i>	1 (1.5)	0.02 \pm 0.1	1	3 (50)	1 \pm 1.5	2 \pm 1.7 (1-4)	SI
Unidentified immature trematode	3 (5)	2 \pm 9	42.3 \pm 3 (39-45)	absent	absent	absent	SI
Unidentified metacercariae	5 (8)	1.3 \pm 8.7	16.8 \pm 29.6 (1-69)	1 (17)	1 \pm 2.4	6	LM, BC
Cestodea							
Unidentified plerocercoid	1 (1.5)	0.2 \pm 1.7	14	absent	absent	absent	BC, L
Unidentified cestode cyst	1 (1.5)	0.1 \pm 0.9	7	absent	absent	absent	S
<i>Mesocostoides</i> sp.	8 (12)	NC	NC	absent	absent	absent	BC, SI, LI, LV, M
Nematoda							
<i>Cosmoecoides variabilis</i>	14 (22)	0.7 \pm 3	3.3 \pm 5 (1-23)	4 (67)	1 \pm 0.9	1.5 \pm 0.6 (1-2)	SI, LI, L
<i>Oswaldocruzia pipiens</i>	4 (6)	0.08 \pm 0.3	1.3 \pm 0.5 (1-2)	absent	absent	absent	SI

* Number (%) infected.

† BC = body cavity; L = lungs; LI = large intestine; LM = leg muscles; LV = liver; M = musculature; S = stomach; SI = small intestine; UB = urinary bladder.

‡ NC = not counted.

helminth abundance for all worm species that could be counted accurately in the treefrogs was 4.4 ± 12.9 . Of the 5 (83%) infected chorus frogs, 2 harbored 1 species, 2 harbored 2 species, and 1 harbored 3 species. Prevalence and mean intensities were highest for *Cosmocercoides variabilis* Harwood, 1930, and *G. pennsylvaniensis*. Overall mean helminth abundance in the chorus frogs was 3.0 ± 3.3 . There was no significant correlation ($P > 0.05$) between helminth abundance for any of the worm species that could be counted accurately and WW or SVL for treefrogs or chorus frogs. Twenty-nine (45%) treefrogs had identifiable stomach contents, but only 2 chorus frogs had stomach contents. The treefrog diet consisted of insects, with coleopterans making up 43% of the diet followed by lepidoptera larvae (24%), unidentifiable insects (22%), and orthopterans (11%). The stomachs of the 2 chorus frogs contained coleopterans and unidentifiable arthropods.

The protozoan *Opalina* sp. Purkinje and Valentin, 1840, was found in the small and large intestine of the treefrogs. These protozoans were not identified to species because of lack of range of forms as suggested by Sandon (1976). *Opalina* spp. were previously reported in *H. chrysoscelis* from Texas by Metcalf (1923) and the sister species *H. versicolor* from various locations in the midwestern and southern parts of the United States. The absence of *Opalina* sp. from the western chorus frogs may be due to the small sample of frogs collected. A number of previous reports have shown that the western chorus frog and other *Pseudacris* species harbor these protozoans (Metcalf, 1923; Brandt, 1936; Odlaug, 1954; McAllister, 1987, 1991).

Nyctotherus cordiformis Ehrenberg, 1838, infected both host species in this study. These ciliates are common parasites of *Hyla* and *Pseudacris* (Brandt, 1936; McAllister, 1987, 1991; McAllister et al., 1993) and have been reported previously from *H. versicolor* from a number of locations throughout its range, although the prevalence reported was much higher than that in this study (Wichterman, 1936; Campbell, 1968). *Hyla chrysoscelis* is a new host record for this ciliate. Prevalence of infection in this hybrid species was more comparable to that of terrestrial anurans such as *Bufo* (Campbell, 1968; McAllister et al., 1989) and may reflect differences in habitat utilization from *H. versicolor*. Blair (1958) reported that *H. chrysoscelis*

is a grassland species in parts of its range, whereas in Wisconsin its distribution in general corresponds to prairies, oak savannas, and pine savannas (Jaslow and Vogt, 1977). Another explanation for the low prevalence observed may be the time of year these frogs were collected. Brandt (1936) reported low prevalence of this ciliate in *Pseudacris* and *Hyla* during early summer.

Polystoma nearcticum Paul, 1938, infected the urinary bladder of *H. chrysoscelis*. This species has been reported previously from *H. versicolor* from Minnesota and *H. cinerea* Schneider, 1799, the green treefrog, from Florida (Paul, 1938). Brooks (1976), in his survey of Nebraska amphibians, found no *H. chrysoscelis* infected with this monogenean. *Hyla chrysoscelis* is a new host record and Wisconsin is a new locality record for *P. nearcticum*.

Three chorus frogs and 1 treefrog were infected with *G. pennsylvaniensis*. This trematode has been reported from Wisconsin spring peepers, *P. c. crucifer* Wied, 1839, by Coggins and Sajdak (1982) and Yoder and Coggins (1996). This is the first report from Wisconsin chorus frogs and the first report from Cope's gray treefrogs.

Three treefrogs were infected with an unidentified immature digenean located in the small intestine. These trematodes were small and showed some development of testes and ovary, but vitellaria, genital pore, or uterus could not be seen. Both frog species were infected at low prevalence with an unidentified metacercaria located in the leg muscles and body cavity. Metacercariae have previously been reported at low prevalence from leg muscle and body cavity musculature in other *Hyla* and *Pseudacris* species (Ulmer, 1970; Yoder and Coggins, 1996).

Ten treefrogs were infected with numerous metacestodes. Eight of the 10 treefrogs were heavily infected with tetrathyridia of *Mesocostoides* sp. Valunt, 1863. These organisms were encapsulated in the intestine, liver, and musculature, but a few were also found free in the body cavity. Three of the treefrogs harbored heavy infections under the skin of the hind legs, with as many as 300 metacestodes/leg. These treefrogs displayed small abrasions on the outer surface of the skin, which appeared red or pink in color instead of the usual cream white color. Although a number of bufonids and ranids have been reported to be infected with *Mesocostoides*

sp. (see McAllister et al., 1989; McAllister and Conn, 1990; McAllister et al., 1995) only one hylid is known to be infected with this metacestode (McAllister, 1987). Therefore, Cope's gray treefrog is a new host record. Two other Cope's gray treefrogs were infected with other unidentified metacestodes; 1 treefrog harbored 14 plerocercoids, 13 in the body cavity and 1 in a lung. All metacestodes possessed a tetracetabulate scolex with an apical organ. The other treefrog possessed 7 cestode cysts on the outer mesentery of the stomach. These cestodes lacked an excretory antrum and did not appear to be *Mesocostoides* sp. Plerocercoids and other cestode cysts have been previously reported from *H. versicolor* in Missouri and other hylids from North Carolina (Brandt, 1936; Shannon, 1988).

Two species of nematodes, *Oswaldocruzia pipiens* Walton, 1929, and *Cosmocercoides variabilis* Harwood, 1930, infected Cope's gray treefrog, and *C. variabilis* infected the western chorus frogs. Four male and 1 gravid female *O. pipiens* were found in the small intestine of 4 treefrogs. Cope's gray treefrog is a new host record for *O. pipiens*.

A total of 46 *C. variabilis* (16 males, 20 females, and 10 J₄ larvae) were recovered from 14 of the treefrogs, and 6 (1 male, 3 females, and 2 J₄ larvae) were recovered from 4 of the chorus frogs. Vanderburgh and Anderson (1987a) reported that *C. variabilis* is a parasite of amphibians but *C. dukae* Holl, 1928, is a parasite of terrestrial molluscs, with inadvertent occurrences in animals that feed upon terrestrial molluscs (Anderson, 1960). The major difference in the 2 nematode species is the number of rosette papillae per subventral row in males; male *C. dukae* have 9–21 rosette papillae (averaging 13 or 14) and *C. variabilis* have 15–25 (averaging 20 or 21). All males in the present study possessed 17–23 rosette papillae, averaging 19.6. Measurements of gubernaculum length of males and esophagus length and bulb width for males and females fell in the ranges for those measurements in *C. variabilis* as given by Vanderburgh and Anderson (1987a) for toads. The J₄ larvae were also located in the lungs and small intestine, and all adult females were gravid with developing larvae in the eggs. The diet of Cope's gray treefrog, as indicated by this study and previous work (Ralin, 1968), consists mostly of insects, and molluscs probably play an insignificant role if any in their diet. Therefore, the

specimens collected in the present study probably are *C. variabilis*. This nematode has a direct life cycle that includes skin penetration, molting in the lungs or body cavity, and maturing in the intestine (Baker, 1978c). It was suggested that this parasite may be restricted to certain amphibian groups such as hylids, microhylids, and bufonids (Vanderburgh and Anderson, 1987a). *Cosmocercoides variabilis* is a common parasite of the eastern American toad *Bufo a. americanus* Holbrook, 1836 (Vanderburgh and Anderson, 1987a, b; Joy and Buntun, 1997), and has previously been reported from the western chorus frog in Canada (Vanderburgh and Anderson, 1987a). This is the first report from Cope's gray treefrog and the first report from Wisconsin chorus frogs.

The breeding pond probably serves as the most important focus of infection with these endoparasite species, and diet of adult frogs plays a lesser role. Brandt (1936) suggested that an arboreal habitat is less conducive to metazoan parasitism than are terrestrial or aquatic habitats. The arboreal nature of Cope's gray treefrogs probably has an effect on parasite colonization during the tadpole stage or breeding period of these frogs. Of the 11 endoparasites recovered, 6 have been reported to have their life cycles synchronized to the amphibian tadpole stage and their emergence period from the pond (Brandt, 1936; Wichterman, 1936; Paul, 1938; Cheng, 1961; El Mofty and Smyth, 1964; El Mofty, 1973; Sullivan and Byrd, 1970; Baker, 1978a). The 3 metacestode species recovered are probably acquired by frogs feeding on intermediate hosts. Stomach content analysis revealed that the diet of these frogs is less diverse than that of other Wisconsin anurans (Vogt, 1981). Accordingly, endoparasites dependent on intermediate hosts were found at a lower prevalence in these hosts than in other Wisconsin frogs (Williams and Taft, 1980; Coggins and Sajdak, 1982; Yoder and Coggins, 1996).

Results of the current survey support previous work on treefrog endoparasites, indicating that most are not host specific. In the present study, both frogs utilized the same breeding ponds, with the western chorus frog breeding from late March to late June and Cope's gray treefrog breeding from mid May to late June (pers. obs.). Because of the overlap in habitat utilization by adults and tadpoles of these hosts, transmission of parasites between them is likely. Although

prevalence, mean intensity, and mean abundance was not compared among the two host species because of the low number of chorus frogs examined, the 2 hosts shared 4 endoparasite species, and 2 other species found in Cope's gray treefrogs have been previously reported in western chorus frogs (Metcalf, 1923; Baker, 1977). *Polystoma nearcticum* is the only parasite in this study that is host specific to the genus *Hyla* and has not been reported in *Pseudacris* (see Paul, 1938; Campbell, 1968). Therefore, despite the occurrence of some parasite species in certain amphibian hosts, if the opportunity arises, some parasites are capable of infecting several different host species, confirming that ecological influences can affect host specificity (Prudhoe and Bray, 1982).

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