Parasites of the Mink Frog (Rana septentrionalis) from Minnesota, U.S.A.

ANNA M. SCHOTTHOEFER,^{1,5,6} MATTHEW G. BOLEK,² REBECCA A. COLE,³ AND VAL R. BEASLEY⁴

¹Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61802, U.S.A.

(e-mail: gve3@cdc.gov),

²Department of Zoology, Oklahoma State University, Stillwater, Oklahoma 74078, U.S.A. (e-mail: bolek@okstate.edu),

³USGS National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, U.S.A.

(e-mail: RCole@usgs.gov), and

⁴ Department of Veterinary Biosciences, University of Illinois at Urbana–Champaign, 2001 S Lincoln Ave., Urbana, Illinois 61802, U.S.A. (e-mail: val@illinois.edu)

ABSTRACT: Twenty-two mink frogs, *Rana septentrionalis*, collected from two locations in Minnesota, United States, were examined for helminth and protozoan blood parasites in July 1999. A total of 16 parasite taxa were recovered including 5 larval digenean trematodes, 7 adult digenean trematodes, 3 nematodes, and 1 *Trypanosoma* species. Infracommunities were dominated by the digeneans in terms of richness and abundance. In particular, echinostomatid metacercariae in the kidneys of frogs were the most common parasites found, infecting 100% of the frogs and consisting of about 90% of all helminth individuals recovered. *Gorgodera amplicava, Gorgoderina multilobata, Haematoloechus parviplexus, Haematoloechus breviplexus, Cosmocercoides dukae*, and *Oswaldocruzia pipiens* represent new host records. The survey presented here represents the second known helminth survey of mink frogs conducted in North America. A summary of metazoan parasites reported from mink frogs is included.

KEY WORDS: Rana septentrionalis, mink frog, helminths, Minnesota, echinostomatid, Fibricola, plagiorchiid, Apharyngostrigea pipientis, Haematoloechus parviplexus, Haematoloechus longiplexus, Haematoloechus breviplexus, Gorgodera amplicava, Gorgoderina multilobata, Cephalogonimus americanus, Loxogenes arcanum, Oswaldocruzia pipiens, Cosmocercoides dukae, Trypanosoma pipientis.

The mink frog (*Rana sepentrionalis*) is a north temperate frog species, ranging in Canada from Manitoba to Labrador and the Maritime Provinces, south along the St. Lawrence River to northern New York, and west to south-central Minnesota in the United States (Casper, 2005). It is a member of the *Aquarana* Dubois 1992 clade (also known as the *Rana catesbeiana* group) as defined by Hillis and Wilcox (2005). The mink frog's closest extant relatives include the pig frog (*Rana grylio*), bullfrog (*Rana catesbeiana*), and green frog (*Rana clamitans*) (see Austin et al., 2003; Hillis and Wilcox, 2005). It is a highly aquatic frog, with a 1–2 year larval period (Casper, 2005).

Bacterial (*Aegyptianella ranarum* [Desser, 1987], *Aeromonas* spp. [Hedeen, 1972a]), viral (icosahedral viruses [Gruia-Gray et al., 1989; Desser, 1992]), and fungal (*Batrachochytrium dendrobatidis* [Longcore et al., 2007]) pathogens have been reported from mink frogs. Protozoan parasites, including intestinal coccidia, intracellular blood parasites, and trypanosomes, have also been reported (Werner and Walewski, 1976; Barta and Desser, 1984; Chen and Desser, 1989; Jones and Woo, 1989). However, there have been few reports of helminth parasites. Mace and Anderson (1975) reported that mink frogs were a paratenic host for the nematode, Dioctophyme renale, and Barta and Desser (1984) reported microfilariae of Foleyella sp. (currently designated as Waltonella sp. [Bain and Prod'hon, 1974]) in the blood. Sutherland (2005) and Wilson et al. (2005) also reported mink frogs as hosts for the metacercariae of Ribeioria ondatrae, which have been implicated in causing limb deformities in amphibians in North America (Johnson et al., 1999; Johnson et al., 2002) and, indeed, Sutherland (2005) found infections associated with limb deformities in mink frog populations of Minnesota. Conn et al. (2002) also reported mink frogs from upstate New York as hosts for echinostomatid metacercariae. The only known published survey of helminth parasites was conducted in 1948-1949 in northern Maine by Bouchard (1951). Here, we contribute to the known records of mink frog helminth parasites by reporting the results of a survey of parasites in frog samples from two locations in south-central Minnesota, United States.

⁵Current address: Centers for Disease Control and Prevention, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Division of Vector-Borne Infectious Diseases, 3150 Rampart Road, Fort Collins, Colorado 80521, U.S.A. (gve3@cdc.gov)

⁶Corresponding author.

MATERIALS AND METHODS

Mink frogs (n = 22) were collected in July 1999 at two wetlands in south-central Minnesota, U.S.A. Site 1 was located in Stearns County at Birch Lake State Forest (45°46'14.74"N, 94°47'17.41"W). Site 2 was a privately owned wetland in Todd County (45°48'23.33"N, 94°50'42.61"W). Captured frogs were held on ice and transported from Minnesota to Wisconsin, where they were held at 10°C until the time of necropsy (up to 6 d postcapture). After euthanasia by immersion in 1:500 tricaine methanesulfonate (MS-222), frogs were weighed to the nearest 0.01 g and their snout-vent lengths were recorded (mm). Each frog was examined for ectoparasites and external abnormalities prior to necropsy. Blood films were collected and fixed in 100% methanol for later examination of blood parasites. The buccal and body cavities, internal organs, eyes, and skin were examined under stereomicroscopy for helminth parasites following standard protocols (Smyth and Smyth, 1980; Pritchard and Kruse, 1982). Parasite specimens recovered were isolated in amphibian's ringer solution (Smyth and Smyth, 1980) in small watch glasses, counted, and fixed in hot, 10% neutralbuffered formalin (NBF) or 95% ethyl alcohol. Trematodes were prepared for identification using Semichon's acetocarmine stain, and nematodes were cleared in glycerine (Pritchard and Kruse, 1982). Blood films were stained with Giemsa and examined under ×400 magnification; the number of fields of view inspected approximated 100,000 erythrocytes per frog. The carcasses of frogs were fixed in 10% NBF and cleared and stained using a modified method of Hanken and Wassersug (1981) for later identification and enumeration of encysted metacercariae in the musculature. This method has previously been used to detect and quantify metacercariae embedded in the musculature of amphibians (Sessions and Ruth, 1990). Recognition of the different metacercariae observed during necropsies was possible in the cleared and stained frogs due to the different staining patterns and other morphological characteristics that were preserved in the specimens (e.g., excretory vesicles, echinostomatid collar spines; A. M. Schotthoefer, personal observation). Identifications of adult taxa were made using original descriptions of parasites and related literature (Olsen, 1937; Lang, 1968; Ulmer, 1970; Baker, 1977; Schell, 1985; Vanderburg and Anderson, 1987a, 1987b, 1987c; McAlpine and Burt, 1998; Bolek and Janovy, 2007). Additionally, the paratype of Gorgoderina multilobata was borrowed from the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (HWML 23428) and compared to worms recovered from our study. Larval digeneans were identified based on descriptions in Chandler (1942), Ulmer (1970), Schell (1985), McAlpine and Burt (1998), and Sutherland (2005) and, in some cases, were identified only to order. Descriptions in Woo (1969) were used to identify the trypanosome species. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln (collection nos. HWML): Gorgoderina multilobata (49009), Gorgodera amplicava (49010), Cephalogonimus americanus (49011), Haematoloechus longiplexus (49012), Haematoloechus parviplexus (49013), Haematoloechus breviplexus (49014), Loxogenes arcanum (49015), undetermined echinostomatid (49016), Cosmocercoides dukae (49017-49018), and Oswaldocruzia pipiens (49019). Cleared and stained frogs were deposited in the Bell Museum of Natural

History, University of Minnesota, Minneapolis–St. Paul, Minnesota (Collection nos. 14624–15168).

Prevalences, mean abundances, and intensities (sensu Bush et al., 1997) were calculated for each parasite taxa recovered. We also determined the taxa that dominated each infracommunity in terms of representing more than 50% of the individuals recovered per host.

RESULTS

Six (5 males:1 female) and 16 (9 males:7 females) mink frogs were collected and necropsied from Sites 1 and 2, respectively. The average weights and lengths (± 1 SD) of frogs collected from Site 1 were 7.2 (3.72) g and 44.5 (6.92) mm, respectively, and from Site 2 were 7.8 (0.93) g and 46.1 (2.09) mm, respectively. All of the frogs examined were infected with at least one species of parasite. In total, 16 parasite taxa were recovered from the frogs examined; 5 of these taxa were larval digenean trematodes, 7 were adult digenean trematodes, 3 were nematodes, and 1 Trypanosoma species was detected in the blood (Table 1). Eleven taxa were found at Site 1, and 14 were found at Site 2. On average (± 1 SD), frogs were infected with 4.0 (2.76) parasite taxa at Site 1 and 4.3 (1.61) taxa at Site 2.

Infracommunities ranged from 56 to 208 helminth individuals per frog at Site 1 and from 69 to 979 per frog at Site 2. Larval trematodes dominated the communities; approximately 90% of the helminths recovered at Site 1 were larval trematodes, as were about 96% of the helminths recovered at Site 2. Echinostomatid metacercariae from the kidneys of frogs had the highest infrapopulations (Table 1) and dominated the infracommunities in 19 of the 22 (86.4%) frogs examined. A metacercaria, identified as belonging to the Order Plagiorchiida, and found encysted in the body cavity on surfaces of visceral organs and in the musculature (referred to here as Plagiorchiid2), dominated the infracommunities of 3 of the 6 (50%) frogs necropsied from Site 1. On average (± 1 SD), frogs were infected with 1.5 (0.54) and 2.5 (0.73) larval trematode taxa, 1.5 (1.64) and 1.3 (1.00) adult trematode taxa, and 0.7 (0.52) and 0.4 (0.63) nematode taxa at Sites 1 and 2, respectively. In general, adult trematode and nematode infrapopulations did not exceed 35 and 7 worms, respectively (Table 1).

DISCUSSION

The mink frog is a highly aquatic frog. Adults occur in permanent water bodies, especially where there are protected areas with abundant aquatic macrophytes (Hedeen, 1986; Casper, 2005). Individ-

	S	ite 1 $(n = 6)$		S	Site 2 $(n = 16)$			
Parasite taxa (stage*)	Prevalence (%)	Mean abundance (±1 SD)	Range of intensities	Prevalence (%)	Mean abundance (±1 SD)	Range of intensities	Site(s) of infection	
Protozoa								
Trypanosoma pipientis	33.3		1-12†	6.3		1	Blood	
Trematoda								
Echinostomatids (L)‡	100	61.3 (57.59)	6-147	100	379.9 (265.78)	40-972	Kidneys	
Fibricola sp. (L)	0			62.5	7.1 (12.56)	1-51	Body cavity, musculature	
Apharyngostrigea pipientis (L)	0			6.3	0.07 (0.25)	1	Body cavity	
Unidentified Plagiorchiid1 (L)	0			50	2.5 (3.72)	1–12	Body cavity, musculature, under skin	
Unidentified Plagiorchiid2 (L)	50	54.5 (72.88)	65–185	31.3	0.8 (1.44)	1–5	Body cavity, musculature, under skin	
Haematoloechus longiplexus (A)	16.7	0.3 (0.82)	2	37.5	2.1 (3.86)	1-11	Lungs	
Haematoloechus parviplexus (A)	33.3	2.3 (4.80)	2-12	25	2.4 (6.87)	1–27	Lungs	
Haematoloechus breviplexus (A)	0			6.3	0.06 (0.25)	1	Lungs	
Haematolechus sp. (immature A)	16.7	0.2 (0.41)	1	25	0.7 (1.45)	1–5	Lungs	
Cephalogonimus americanus (A)	50	6 (8.00)	4-18	0			Intestine	
Loxogenes arcanum (A)	16.7	3.3 (8.16)	20	0			Wall of stomach at pylorus, pancreas, surface of liver	
Gorgodera amplicava (A)	0			25	0.4 (0.74)	1-2	Bladder	
Gorgoderina multilobata (A)	16.7	0.2 (0.41)	1	6.3	0.07 (0.26)	1	Bladder	
Unidentified gorgoderid (A)	0			6.3	0.07 (0.26)	1	Bladder	
Nematoda								
Oswaldocruzia pipiens (A)	16.7	1.2 (2.86)	7	6.3	0.2 (0.75)	3	Intestine, body cavity	
Cosmocercoides dukae (A)	33.3	1.2 (1.83)	3–4	25	0.4 (0.72)	1-2	Rectum	
Unidentified nematode (J)	16.7	0.3 (0.82)	2	12.5	0.3 (0.77)	1-3	Intestine	

Table 1.	Prevalence,	mean	abundance,	and	range	of	intensities	of	parasites	found	in	22	specimens	of	Rana
septentrio	nalis from Mi	nnesot	ta, United St	ates.											

* Parasite stage recovered; L, larvae; J, juvenile; A, adult; includes gravid and non-gravid specimens.

† Per 100,000 erthrocytes.

‡ May include members of the genera Echinostoma and Echinoparyphium.

uals are described as spending the majority of their time resting and foraging in water, often in a semisubmerged state, and their gut contents reflect a primarily aquatic diet (Hedeen, 1972b). They have been found in terrestrial habitats only after night rains (Hedeen, 1986). Larvae typically metamorphose after a 1-yr larval period, although 2-yr larval periods do occur (Hedeen, 1972c; Leclair and Laurin, 1996; Casper, 2005). The helminth communities found in the mink frogs from Minnesota were consistent with those previously reported for aquatic frogs. Frog species living a predominately aquatic existence tend to be infected with parasite communities that are dominated by trematode taxa, whereas the communities found in terrestrial species are comprised largely of nematode and, sometimes, cestode taxa (Brandt, 1936; Rankin, 1945; Muzzall, 1991; McAlpine, 1997; Bolek and Coggins, 2003). In the mink frog individuals that we examined, the parasite communities were dominated by trematode species in terms of richness, prevalences, and abundances (Table 1). In particular, larval stages of trematodes were the most numerically abundant and, of the 3 taxonomic groups of helminths detected (e.g., larval trematode, adult trematode, and nematode), adult trematode species were the most species rich. Bouchard (1951) also found a diversity of trematode taxa infecting mink frogs in Maine and found that the prevalences and intensities of these infections were frequently higher in mink frogs than in the more terrestrial species examined from the same region (e.g., *Bufo americanus, Rana pipiens, Rana sylvatica*).

Many of the adult trematode taxa detected in our survey in Minnesota were also found in mink frogs in Maine (Bouchard, 1951; Table 2). However, we did not find *Glypthelmins quieta*, *Haematoloechus medioplexus*, *Haematoloechus varioplexus* (but see note Table 2), *Megalodiscus temperatus*, *Halipegus* sp., or *Cylindrotaenia americana*, as were reported for Maine mink frogs (Bouchard, 1951). The one blood parasite detected in our survey, *Trypanosoma pipientis*, was also previously reported in mink frogs in Ontario, Canada (Barta and Desser, 1984; Table 2); whereas *G. amplicava*, *G. multilobata*, *H. parviplexus*, *H. breviplexus*, *C. dukae*, and *O. pipiens* are new host records.

The helminth communities in mink frogs in Minnesota were comprised of parasite taxa that have frequently been reported to infect other anuran species (e.g., Ulmer, 1970; McAlpine and Burt, 1998). Exceptions are H. parviplexus and L. arcanum. Both of these species were first reported from bullfrogs by Stafford (1900, 1902) and have since been predominantly reported from bullfrogs, green frogs, and mink frogs (Bouchard, 1951; Williams and Taft, 1980; Muzzall, 1991; McAlpine and Burt, 1998; Bolek and Janovy, 2007), although Goldberg and Bursey (2007) recently reported L. arcanum from Rana (=Lithobates) vaillanti in Costa Rica. Bolek and Janovy (2007) demonstrated that H. parviplexus infects bullfrogs and green frogs, but that leopard frogs appear to be resistant to infection with this Haematoloechus species. Our finding of H. parviplexus in Minnesota mink frogs raises the possibility that H. parviplexus is an Aquarana Dubios 1992 specialist.

It is possible that *L. arcanum* also represents a species that diverged and coevolved with the *Aquarana* Dubois 1992 clade. However, because it has also been reported to infect leopard frogs (Osborn, 1912; Crawford, 1938; Goldberg and Bursey, 2007), it is unlikely that it is an *Aquarana* specialist. Interestingly, however, we did not detect this species in over 500 leopard frogs examined from the same region, and during the same time period, as the mink frogs that were examined for the current study (Schotthoefer, 2003). Adults of *L. arcanum* are found encysted in the wall of the duodenum at the pylorus, although they may also be found associated with the liver or urinary bladder (Stafford, 1900;

Osborn, 1912; Crawford, 1938). Metacercariae of *L. arcanum* are transmitted to frogs in odonate naiads (Crawford, 1938).

Mink frog populations have recently been reported with high prevalences of limb abnormalities (Gardiner and Hoppe, 1999; Vandenlangenberg et al., 2003; Hoppe, 2005), and Sutherland (2005) found abnormalities associated with *R. ondatrae* infections. In the mink frog individuals we necropsied, no *R. ondatrae* infections were detected, and only 5 of 144 (3.5%) mink frogs captured and examined for external abnormalities during a 3-yr study of 36 wetlands, including the 2 studied here, were observed with abnormalities (Schoff et al., 2003). Therefore, it does not appear that mink frogs in the region of Minnesota we studied are experiencing population constraints related to high abnormality rates.

In summary, mink frogs are somewhat unique among the ranid species of North America in having a range restricted to the high northern latitudes. The species is also described as being one of the most aquatic frogs in North America (Hedeen, 1986; Casper, 2005). Surprisingly, the helminth communities of the species have been little studied. Our examination reveals a helminth community dominated by larval and adult digenean trematodes, with the majority of species being shared with other ranid species. Comparative studies of the helminth communities of the mink frog, and its close relatives bullfrogs and green frogs, would provide insight into the roles of contemporary ecology and evolution in structuring anuran parasite communities in temperate climates.

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Parasite	% Prevalence (number of hosts examined)	Location	Reference		
	(number of nosis enumied)	Locaton	10101000		
Protozoa					
Trypanosoma pipientis	2.7 (75)	Ontario, Canada	Barta and Desser (1984)		
	43.5 (46)	Guelph and Ontario, Canada	Jones and Woo (1989)		
	13.6 (22)	Minnesota	This study		
Trypanosoma rotatorium	25.3 (75)	Ontario, Canada	Barta and Desser (1984)		
	8.7 (23)	Michigan	Werner and Walewski (1976)		
Trypanosoma ranarum	2.2 (46)	Guelph and Ontario, Canada	Jones and Woo (1989)		
	4.3 (23)	Michigan	Werner and Walewski (1976)		
Hepatozoon sp.	8.0 (75)	Ontario, Canada	Barta and Desser (1984)		
Lankesterella minima	20.0 (75)	Ontario, Canada	Barta and Desser (1984)		
Babesiasoma stableri	18.6 (75)	Ontario, Canada	Barta and Desser (1984)		
Eimeria algonquini	10.3 (68)	Ontario, Canada	Chen and Desser (1989)		
Eimeria kermiti	1.5 (68)	Ontario, Canada	Chen and Desser (1989)		
Frematoda					
Echinostomatid metacercariae	100.0 (22)	Minnesota	This study		
	100.0 (2)	New York	Conn et al. (2002)		
Fibricola sp. metacercariae	45.5 (22)	Minnesota	This study		
Apharyngostrigea pipientis	4.5 (22)	Minnesota	This study		
metacercariae					
Plagiorchiid metacercariae	72.7 (22)	Minnesota	This study		
Ribeiroia ondatrae metacercariae	NR‡	Minnesota	Sutherland (2005)		
	NR	Wisconsin	Wilson et al. (2005)		
Cephalogonimus americanus	10.5 (57)	Maine	Bouchard (1951)		
	13.6 (22)	Minnesota	This study		
Glypthelmins quieta	40.4 (57)	Maine	Bouchard (1951)		
Gorgoderina attenuata	29.8 (57)	Maine	Bouchard (1951)		
Gorgoderina simplex	8.8 (57)	Maine	Bouchard (1951)		
Gorgoderina translucida	7.0 (57)	Maine	Bouchard (1951)		
Gorgoderina multilobata	9.1 (22)	Minnesota	This study		
Gorgodera amplicava	18.2 (22)	Minnesota	This study		
Loxogenes arcanum	14.0 (57)	Maine	Bouchard (1951)		
	4.5 (22)	Minnesota	This study		
Megalodiscus temperatus	3.5 (57)	Maine	Bouchard (1951)		
Haematoloechus longiplexus	42.1 (57)	Maine	Bouchard (1951)		
	31.8 (22)	Minnesota	This study		
Haematoloechus medioplexus	10.5 (57)	Maine	Bouchard (1951)		
Haematoloechus varioplexus†	5.3 (57)	Maine	Bouchard (1951)		
Haematoloechus parviplexus	27.3 (22)	Minnesota	This study		
Haematoloechus breviplexus	4.5 (22)	Minnesota	This study		
Halipegus sp.	28.1 (57)	Maine	Bouchard (1951)		
Cestoda					
Cylindrotaenia americana	12.3 (57)	Maine	Bouchard (1951)		
Nematoda					
Oswaldocruzia pipiens	9.1 (22)	Minnesota	This study		
Cosmocercoides dukae	27.3 (22)	Minnesota	This study		
Waltonella sp. microfilaria	1.3 (75)	Ontario, Canada	Barta and Desser (1984)		
Dioctophyme renale juveniles	9.6 (146)	Ontario, Canada	Mace and Anderson (197		

Table 2. Summary of the parasites of Rana septentrionalis in North America.*

* Does not include intestinal protozoa reported by Camara and Buttrey (1961).

 \dagger Bouchard reported *H. similiplexus* in mink frogs, which is a synonym of *H. varioplexus*. However, it is unclear if Bouchard (1951) was dealing with *H. varioplexus* or *H. parviplexus* because no voucher specimens exist and *H. parviplexus* was originally described as *H. varioplexus*, and these names have been used interchangeably in the literature (see Bolek and Janovy, 2007).

‡NR, not reported.

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