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DISTRIBUTION AND REPRODUCTIVE STRATEGIES OF *GYRINICOLA BATRACHIENSIS* (OXYUROIDEA: PHARYNGODONIDAE) IN LARVAE OF EIGHT SPECIES OF AMPHIBIANS FROM NEBRASKA

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ABSTRACT: In total, 462 tadpoles and salamander larvae of 8 species were examined for the presence of *Gyrinicola batrachiensis* from 5 locations in Nebraska. Infection by *G. batrachiensis* occurred in tadpoles of *Rana blairi*, *Rana catesbeiana*, *Rana pipiens*, and *Bufo woodhousii*. Tadpoles of *Hyla chrysoscelis*, *Spea bombifrons*, and *Pseudacris maculata* and larvae of *Ambystoma mavortium* were not infected with *G. batrachiensis*. Population structure, defined as prevalence, mean abundance, and mean intensity of *G. batrachiensis*, varied among tadpoles of different amphibian species and was determined by collection locality, developmental period of tadpole hosts, amphibian species co-occurrence, and different reproductive strategies of *G. batrachiensis*, or a combination. *Gyrinicola batrachiensis* observed in all ranid tadpoles and *B. woodhousii* tadpoles from where bufonids were the only anuran species present, confirmed to the didelphic haplodiploidy and monodelphic parthenogenetic reproductive strategies, respectively. However, tadpoles of *B. woodhousii* that co-occurred with tadpoles of *R. pipiens* at Cedar Creek were inconsistent with these predictions and contained both male and didelphic female nematodes, but at a low mean intensity (1.61 ± 0.70). Didelphic female nematodes from *B. woodhousii* tadpoles at Cedar Creek only produced thick-shelled eggs, whereas nematodes in *R. pipiens* tadpoles had a high mean intensity (14.88 ± 23.83) from this location and contained both thick-shelled and thin-shelled eggs in their respective uteri. More importantly, adult female nematodes from tadpoles of *R. pipiens* and *B. woodhousii* from Cedar Creek were morphologically more similar to each other than to female nematodes recovered from tadpoles of other anuran species, other locations, or both. These data suggest that when strains of *G. batrachiensis* are shared by tadpoles of different amphibian species that differ in developmental period, the nematodes have an intermediate reproductive strategy in amphibian species, with tadpoles having short development.

Species of *Gyrinicola* Yamaguti, 1938 occur in tadpole stages of Holarctic and Neotropical anurans. Currently, 5 species have been described, including *Gyrinicola batrachiensis* (Walton 1929), Adamson 1981, from North America; *Gyrinicola tha* (Dinnik 1933) and *Gyrinicola chabadamsoni* Planade and Baine, 2008 from Europe; *Gyrinicola japonica* Yamaguti 1938 from Japan; and *Gyrinicola chabaudi*, Araujo and Artigas, 1982, from South America. These pinworms infect the gastrointestinal tract of tadpoles, whereas metamorphosing tadpoles and adult frogs are resistant to infections (Adamson, 1981a; Bursey and DeWolf, 1998; Pryor and Greiner, 2004). *Gyrinicola batrachiensis* is considered to have a mutualistic relationship with their tadpole hosts, by increasing fermentation rates within tadpoles and consequently accelerating tadpole developmental time to metamorphosis (Pryor and Bjorndal, 2005).

Adamson (1981a, 1981b, 1981c, 1981d) described the morphology, life cycle, genetics, and seasonal recruitment of *G. batrachiensis* in tadpoles of 8 species of anurans from Canada. His studies demonstrate that *G. batrachiensis* is haplodiploid and that females possess 2 sets of chromosomes (2n), whereas males possess 1 set of chromosomes (n). *Gyrinicola batrachiensis* has a direct life cycle, and tadpoles acquire initial infections by ingesting thick-shelled eggs that are distributed on the pond bottom. Female worms have a complex reproductive anatomy. One uterine horn produces thick-shelled unembryonated eggs used as transmission agents from tadpole to tadpole, whereas the second uterine horn produces thin-shelled eggs with juveniles used for autoinfection. All thin-shelled autoinfective eggs cannot survive outside of the tadpole host and die within an hour in pond water. Importantly, the development of the genital tract producing thin-shelled autoinfective eggs varies according to the amphibian species and its larval developmental time. In some tadpole hosts with short developmental periods (a few weeks), such as *Bufo americanus* and *Bufo terrestris*, *G. batrachiensis* nematodes reproduce parthenogenetically (Adamson, 1981a; Pryor and Greiner, 2004). Female nematodes in these hosts are monodelphic, possessing a single uterine horn that produces thick-shelled environmentally resistant eggs that are shed from the host. In other hosts with longer larval developmental periods (months to years), such as tadpoles of true frogs (*Rana catesbeiana*, *Rana clamitans*, and *Rana pipiens*), both male and female *G. batrachiensis* nematodes occur and reproduce by haplodiploidy (Adamson, 1981a). Female nematodes in tadpoles of these hosts are didelphic, producing thin-shelled autoinfective eggs in 1 uterine horn and thick-shelled environmentally resistant eggs that are shed from the second uterine horn. Adamson's (1981a) experimental infections indicated that tadpoles with long developmental periods, such as *R. clamitans* infected with thick-shelled eggs from naturally infected tadpoles of *B. americanus* with short developmental periods, only produced female worms, whereas tadpoles of *R. clamitans* infected with thick shelled-eggs from naturally infected tadpoles of *R. clamitans* produced both male and female worms. Adamson (1981a) argued that these dramatically different reproductive strategies in *G. batrachiensis* are adaptive responses to different life history and developmental strategies of different anuran hosts. These data suggest that parthenogenetic and haplodiploidy strains of *G. batrachiensis* are genetically determined and that it is unclear whether phenotypic reproductive plasticity exists among these strains.

In North America, *G. batrachiensis* has been reported from tadpoles of 14 anuran species from California, Florida, Michigan, and Ohio, as well as Ontario and Quebec, Canada. However, larvae of most amphibian species from North America have never been examined for this nematode, and, to our knowledge, no infection parameters (prevalence, mean intensity, and mean abundance) or reproductive strategies are available for *G. batrachiensis* in larvae of multiple sympatric amphibian species co-occurring in a single body of water (see Pryor and Greiner, 2004). Here, we examined larvae of 7 species of anurans and 1 species of salamander from 5 locations in Nebraska that differed in their developmental period and amphib-

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TABLE I. Collection site, amphibian species, developmental time (DT) in weeks, prevalence, mean intensity (MI), and mean abundance (MA) of *Gyrinicola batrachiensis* in larvae of 8 species of amphibians collected during 2008 and 2009 from 5 locations in Nebraska.

Collection site	Location	Year	Amphibian species	DT*	% (No. infected/ no. examined)	MI ± 1 SD (range)	MA ± 1 SD
Pawnee Lake	Lancaster County	2008	*Rana catesbeiana*	104–156	33 (1/3)	(21)	7 ± 12.12
	(40.8565N, 96.8927W)	2008	*Rana blairi*	12–24	8 (4/47)	4 ± 4.76 (1–11)	0.34 ± 1.66
		2008	*Hyla chrysoscelis*	4–9.3	0 (0/32)	—	—
		2008	*Pseudacris maculate*	6–13	0 (0/15)	—	—
Beckius Pond	Keith County	2008	*Bufo woodhousii*	5–7	13 (11/85)	1.08 ± 0.51 (1–2)	0.15 ± 0.42
	(41.20835N, 101.61777W)						
Nevens Pond	Keith County	2008	*R. catesbeiana*	104–156	52 (13/25)	8.15 ± 7.21 (1–21)	4.24 ± 6.58
	(41.20710N, 101.40850W)	2009	*R. catesbeiana*	104–156	56 (14/25)	6.78 ± 6.09 (1–16)	3.8 ± 5.65
		2009	*Ambystoma movortium*	8–20	0 (0/50)	—	—
Nevens Pond Puddle	Keith County	2008	*Spea bombifrons*	2–3	0 (0/10)	—	—
	(41.1942N, 101.3892W)	2009	*S. bombifrons*	2–3	0 (0/50)	—	—
Cedar Creek	Keith County	2008	*Rana pipiens*	12–24	10 (4/40)	23.5 ± 36.74 (1–78)	2.35 ± 12.44
	(41.18639N, 101.36276W)	2009	*R. pipiens*	12–24	50 (15/30)	14.88 ± 23.83 (1–74)	7.44 ± 18.19
		2009	*B. woodhousii*	5–7	66 (33/50)	1.61 ± 0.70 (1–3)	1.06 ± 0.96

* Data referenced in Lannoo (2005) or Petranka (1998).

ian species co-occurrence for infections with *G. batrachiensis*. Our goals were to document this nematode in Nebraska, examine field host specificity and reproductive strategy of this nematode in amphibian species that varied in their larval developmental period, and compare the reproductive strategy of this nematode in tadpoles in co-occurring amphibian species with short and long developmental periods in nature.

MATERIALS AND METHODS

During May–July 2008 and 2009, 462 larval amphibians in total, including tadpoles of 7 species of anurans and larvae of 1 species of salamander, were collected from 5 sites in Nebraska (Table I). Larval amphibians were transported to the laboratory in 19-L buckets filled with pond water and killed in MS-222 (tricaine methanesulfonate) within 72 hr of capture. All tadpoles were identified using online keys by Altig et al. (2008), and all larval salamanders were identified based on descriptions in Petranka (1998). During necropsy, complete digestive tracts were removed from tadpoles and salamander larvae and examined for *G. batrachiensis.* All nematodes were removed and fixed in 70% ethanol, cleared in glycerol, and identified as temporary mounts according to Adamson (1981b) and Planade et al. (2008). Pinworms from each amphibian host were sexed and a subset of adult worms from each host species was measured with a calibrated ocular micrometer for total length, maximum width, nerve ring distance from anterior end, esophagus length, pharyngeal bulb length and width, excretory pore distance from anterior end, and tail length. In addition, the location of the vulva from the anterior end and egg length and width of thick-shelled eggs were measured in female worms, and spicule length was measured in male worms (see Fig. 1). All measurements are reported in micrometers, unless otherwise noted. All gravid female *G. batrachiensis* were differentiated as monodelphic or didelphic based on descriptions by Adamson (1981a, 1981b), and the presence of thick-shelled eggs and thin-shelled eggs was recorded for each component population of pinworms for each host and collection year.

Prevalence, mean intensity (MI), and mean abundance (MA) of nematodes for each amphibian species, location, and year was calculated according to Bush et al. (1997). Species-specific larval amphibian developmental times were based on life history descriptions reported in Lannoo (2005) and Petranka (1998). The chi-square test for independence was calculated to compare differences in prevalence, and Student's *t*-test was used to compare differences in mean intensity, mean abundance among tadpoles of the same species collected in multiple years or among tadpoles of multiple species when sample sizes were appropriate (more than 2 individuals infected). Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). A 1-way analysis of variance and Sheffé's post hoc test was used to compare differences of morphological characters of adult male and female worms recovered from tadpoles of different amphibian species. The Kruskal–Wallis test and the Kolmogorov–Smirnov 2-sample post hoc tests were used when variances were heteroscedastic (Sokal and Rohlf, 1981). Voucher specimens for monodelphic and didelphic females, as well as males and juvenile nematodes from larvae of different amphibian species, were deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accessions HWML 66679 juvenile from *B. woodhousii*; 66680 male from *B. woodhousii*; 66681 didelphic female from *B. woodhousii*; and 66682 monodelphic female from *B. woodhousii*; 66683 juvenile from *R. blairi*; 66684 didelphic female from *R. blairi*; 66685 juvenile from *R. catesbeiana*; 66686 male from *R. catesbeiana*; 66687 didelphic female from *R. catesbeiana*; 66688 juvenile from *R. pipiens*; 66689 male from *R. pipiens*; 66690 didelphic female from *R. pipiens*).

RESULTS

Pinworm-infected larval amphibians occurred in Pawnee Lake, Beckius Pond, Nevens Pond, and Cedar Creek, whereas no pinworms occurred in tadpoles from the puddle at Nevens Pond. In total, 622 individual *G. batrachiensis* were recovered from tadpoles of *R. blairi*, *R. catesbeiana*, *R. pipiens*, and *B. woodhousii*, but pinworms were absent from tadpoles of *H. chrysoscelis*, *P. maculata*, and *Spea bombifrons*, and larvae of barred tiger salamanders, *Ambystoma mavortium* (Table I). Prevalence was highest for *B. woodhousii* from Cedar Creek, and mean intensity and mean abundance were highest for *R. catesbeiana* from Nevens Pond and lowest for tadpoles of *B. woodhousii* from Beckius Pond (Table I). No significant differences existed in prevalence, mean intensity, or mean abundance of *G. batrachiensis* from tadpoles of *R. catesbeiana* collected from Nevens Pond during 2008 and 2009 ($\chi^2 = 0.08$, $P > 0.05$; $t = 0.53$, $P = 0.60$ for MI; $t = 0.25$, $P = 0.80$ for MA), whereas prevalence was significantly higher for *G. batrachiensis* from tadpoles of *R. pipiens* collected from Cedar Creek in 2009 than 2008 ($\chi^2 = 13.86$, $P < 0.0001$). There were no significant differences in prevalence or MA of *G. batrachiensis* among co-occurring tadpoles of *R. pipiens* and *B. woodhousii* collected from Cedar Creek ($\chi^2 = 2.00$, $P > 0.05$; $t' = 1.91$, $P > 0.05$), whereas tadpoles of *R. pipiens* had a significantly higher MI (14.88 ± 23.83) of *G. batrachiensis* than tadpoles of *B. woodhousii* (1.61 ± 0.70; $t' = 2.14$, $P = 0.05$) at Cedar Creek.

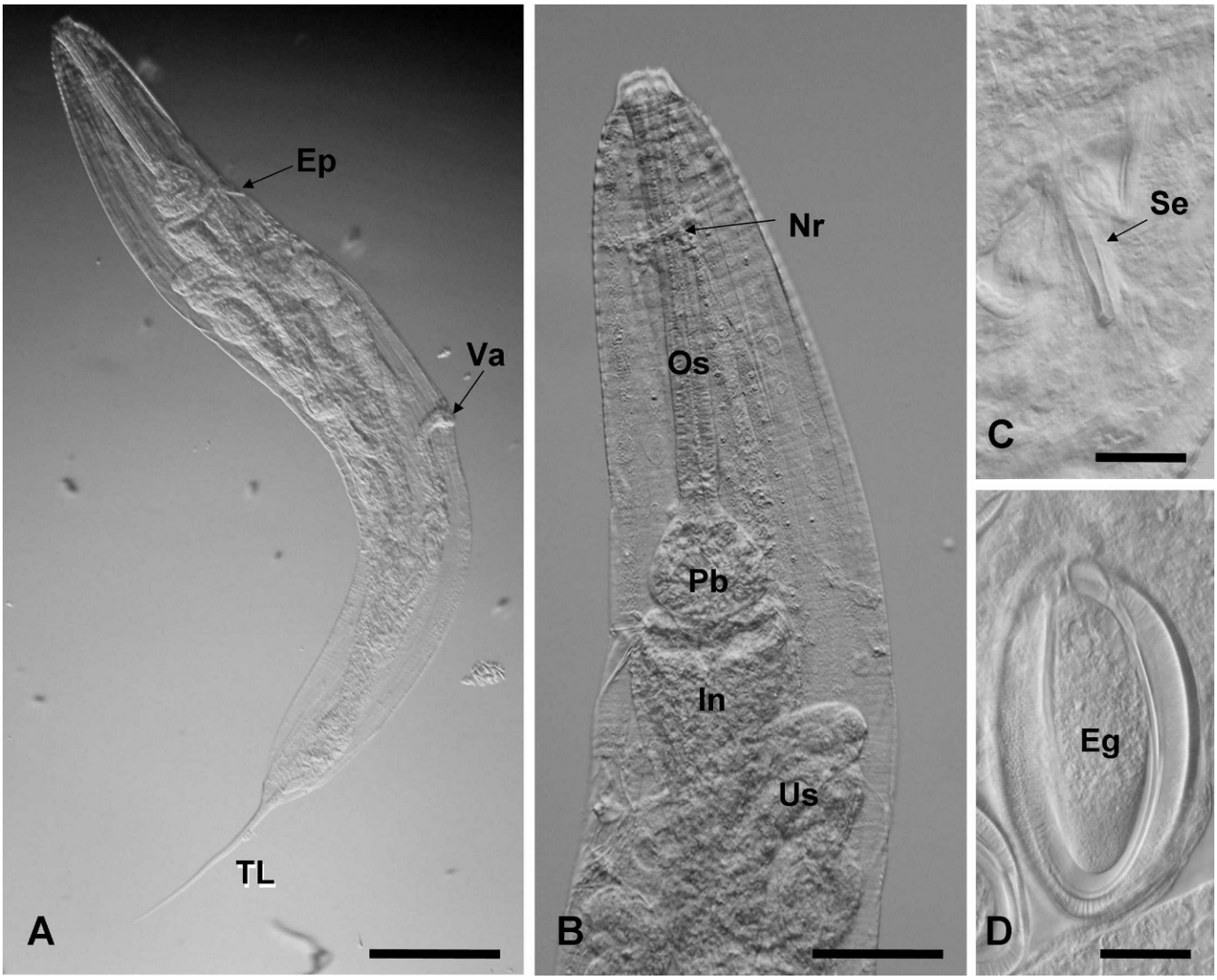

FIGURE 1. Major morphological characters of *Gyrinicola batrachiensis* from a tadpole of *Rana catesbeiana* from Nevens Pond, Keith County, Nebraska. (**A**) Ventral view of entire female worm. Scale bar = 150 μm. (**B**) Anterior end of female worm. Scale bar = 35 μm. (**C**) Lateral view of posterior end of male. Scale bar = 40 μm. (**D**) Thick-shelled egg in uterus. Scale bar = 20 μm. Eg = thick-shelled egg, Ep = excretory pore, In = intestine, Nr = nerve ring, Os = esophagus, Pb = pharyngeal bulb, Se = spicule, TL = tail, Va = vulva, Us =uterus.

At least some female worms with thick-shelled and thin-shelled autoinfective eggs infected tadpoles with a long developmental period, such as *R. blairi* and *R. catesbeiana* from Pawnee Lake, tadpoles of *R. catesbeiana* from Nevens Pond, and tadpoles of *R. pipiens* from Cedar Creek. All gravid females, were didelphic (Fig. 2). Male *G. batrachiensis* were only present in long developing tadpoles of *R. catesbeiana* and *R. pipiens* from Nevens Pond and Cedar Creek, respectively, but they were absent from tadpoles of *R. blairi* and *R. catesbeiana* from Pawnee Lake (Table II). In contrast, only monodelphic females with thick-shelled eggs were recovered from tadpoles of *B. woodhousii* from Beckius Pond, where this was the only amphibian species present. However, both females and male *G. batrachiensis* were recovered from tadpoles of *B. woodhousii* that co-occurred with tadpoles of *R. pipiens* at Cedar Creek (Table II). All gravid female *G. batrachiensis* infecting tadpoles of *B. woodhousii* from Cedar Creek were didelphic, but they only contained thick-shelled eggs (Table II).

Morphological comparisons of adult female and male *G. batrachiensis* from tadpoles of different anuran species and locations indicated that worms were highly variable (Tables III, IV). Female *G. batrachiensis* from different species of hosts exhibited significant differences for all morphological comparisons except for total length, pharyngeal bulb length, vulva distance from the anterior end, and thick-shelled egg length (Table III). Males exhibited significant differences for all comparisons except pharyngeal bulb length and width, and nerve ring and excretory pore distance from the anterior end (Table IV). The Kolmogorov–Smirnov 2-sample tests or Sheffé's post hoc test revealed significant differences in at least 1 of the 9 or 11 morphological characteristics examined in adult male and female *G. batrachiensis*, respectively, among all possible pairs of amphibian species (Tables V, VI). Morphological comparisons of female nematodes recovered from various amphibian hosts indicated that female worms differed significantly in 3 to 5 of the

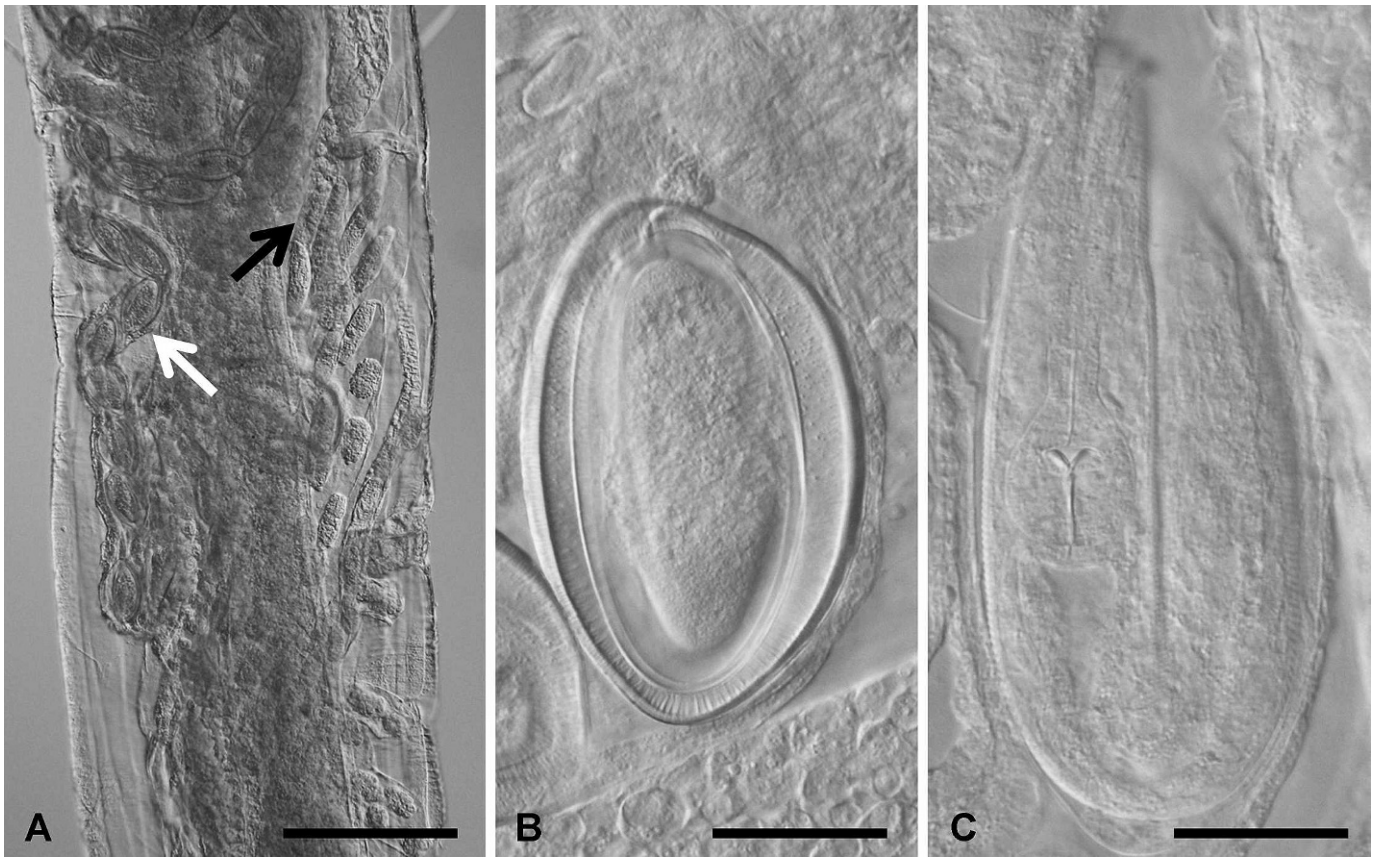

FIGURE 2. Female reproductive system and eggs of *Gyrinicola batrachiensis* removed from a bullfrog tadpole. (**A**) Mid-lateral region of *G. batrachiensis* showing portions of 2 uteri producing thick shelled eggs (white arrow) and thin-shelled eggs (black arrow). Scale bar = 100 μm. (**B**) Thick-shelled egg. Scale bar = 20 μm. (**C**) Thin-shelled egg with developed juvenile. Scale bar = 20 μm.

11 morphological characters measured in all possible anuran species or location pair combinations except for female worms recovered from co-occurring tadpoles of *B. woodhousii* and *R. pipiens* from Cedar Creek, which only differed significantly in tail length (Table V).

DISCUSSION

Our article is the first comparative study of *G. batrachiensis* infections in sympatrically occurring amphibian larvae of different species that differ in their developmental period and co-occurrence. Our data suggest that *G. batrachiensis* populations do not always fit the 2 alternative reproductive strategies in long and short developmental period tadpoles when tadpoles of different amphibian species co-occur. In addition, these results expand the geographic range for *G. batrachiensis* in the Great Plains region of North America and add 2 new host records from tadpoles of *R. blairi* and *B. woodhousii*. Comparative morphological data on adult male and female *G. batrachiensis* from tadpoles of various anuran species indicate that this nematode is highly variable morphologically. However, our measurements of adult female and male *G. batrachiensis* from tadpoles of *R. catesbeiana* and *R. pipiens*, and from tadpoles of *B. woodhousii* from Beckius Pond, fell within 1 SD of the measurements

TABLE II. Number of juveniles, males, and females; uterus type, type of eggs in uterus and suggested reproductive strategy of component populations of *Gyrinicola batrachiensis* recovered from anuran tadpoles from 4 locations in Nebraska.

Location	Amphibian species	Yr	No. of juveniles	No. of males	No. of females	Uterus type	Type of eggs in uterus	Reproductive strategy
Pawnee Lake	*Rana catesbeiana*	2008	0	0	21	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
	Rana blairi	2008	14	0	2	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
Beckius Pond	*Bufo woodhousii*	2008	0	0	14	Monodelphic	Thick-shelled	Parthenogenesis
Nevens Pond	*R. catesbeiana*	2008	31	54	21	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
	R. catesbeiana	2009	27	49	19	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
Cedar Creek	*Rana pipiens*	2008	37	22	35	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
	R. pipiens	2009	61	51	111	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
	B. woodhousii	2009	15	17	21	Didelphic	Thick-shelled	Haplodiploidy

TABLE III. Morphological characters (reported as an average ± 1 SD) of adult female *Gyrinicola batrachiensis* from tadpoles of 4 species of anurans collected from 4 locations in Nebraska.

	Rana blairi (Pawnee Lake)	*Rana catesbeiana* (Nevens Pond and Pawnee Lake)	*Rana pipiens* (Cedar Creek)	*Bufo woodhousii* (Beckius)	*B. woodhousii* (Cedar Creek)	Statistic	*P*
N	2	67	57	11	13		
Total length (mm)	2.93–3.81	2.98 ± 1.11	3.51 ± 2.02	2.45 ± 0.79	3.44 ± 0.89	$H = 4.393$	=0.22
Maximum width	343.6–414.35	376.09 ± 107.10	367.36 ± 142.10	272.86 ± 84.67	296.15 ± 115.29	$H = 11.638$	=0.008
Esophageal length	565.94	441.46 ± 84.67	461.11 ± 97.46	447.42 ± 81.99	337.69 ± 33.25	$H = 23.934$	<0.0001
Pharyngeal bulb length	111.17–112.00	114.60 ± 29.75	113.13 ± 25.27	99.22 ± 17.98	105.39 ± 18.98	$F = 1.363$	=0.347
Pharyngeal bulb width	121.27–141.48	140.40 ± 30.28	119.00 ± 29.66	101.06 ± 20.71	117.69 ± 20.48	$H = 28.028$	<0.0001
Nerve ring*	161.70–222.33	133.30 ± 41.74	163.08 ± 41.09	140.57 ± 23.25	168.33 ± 56.51	$H = 19.111$	=0.0003
Excretory pore*	474.98	720.57 ±339.13	726.69 ± 542.35	658.73 ± 279.58	1,065.83 ± 427.60	$H = 8.442$	=0.0377
Vulva*(mm)	1.32	1.27 ± 0.62	1.54 ± 1.03	1.11 ± 0.33	1.77 ± 0.54	$H = 7.375$	=0.0608
Tail length	485.09–525.51	512.44 ± 281.51	568.62 ± 188.26	362.90 ± 89.76	430.91 ± 187.43	$H = 9.311$	=0.0254
Thick-shelled egg length	83.88 ± 6.48	87.96 ± 9.26	86.06 ± 6.80	87.38 ± 9.77	84.17 ± 5.47	$H = 5.631$	=0.2285
Thick-shelled egg width	57.96 ± 9.40	47.47 ± 9.61	49.73 ± 9.11	41.83 ± 7.75	48.58 ± 10.14	$F = 4.764$	=0.0011

* Distance from anterior end.

provided for *G. batrachiensis* from tadpoles of *R. catesbeiana*, *R. pipiens*, and *B. americanus* from Canada by Adamson (1981b).

Both Adamson (1981a) and Pryor and Greiner (2004) recovered this nematode from ranid, bufonid, and hylid tadpoles, and Pryor and Greiner (2004) also reported this pinworm from tadpoles of a microhylid species. Our study indicates that, in Nebraska, *G. batrachiensis* infected ranid and bufonid tadpoles but that it was absent from tadpoles of *H. chrysoscelis*, *P. maculata*, and *S. bombifrons* and from larvae of *A. mavortium*. However, previous surveys of tadpoles indicate that *G. batrachiensis* has been reported from tadpoles of *P. maculata* and other *Hyla* spp., suggesting that species-specific differences in habitat, behavior, or both among tadpoles of different anuran species may play a role in the observed host specificity of *G. batrachiensis* at Pawnee Lake (Pryor and Greiner, 2004). Field and laboratory studies on the transmission of *G. batrachiensis* by Adamson (1981a, 1981c) clearly indicate that in order for a tadpole to acquire an initial infection of *G. batrachiensis*, they must ingest thick-shelled eggs that are distributed on the pond bottom. Although we have no data on tadpole habitat partitioning among the 4 amphibian species collected from Pawnee Lake, Heyer (1976) examined habitat partitioning among tadpoles of similar anuran species from a pond in Virginia. His study showed that tadpoles of *Pseudacris crucifer* were distributed at the surface of the water column, tadpoles of *H. chrysoscelis* were distributed in mid-water, and tadpoles of *R. clamitans* occupied the bottom of the water column. Together, these data suggest that at Pawnee Lake, tadpoles of *P. maculata* and *H. chrysoscelis* may spend more time at the surface and mid-water compared with benthic-distributed tadpoles such as *R. blairi* and *R. catesbeiana*. These species-specific differences in habitat partitioning may prevent tadpoles of these hylids from coming in contact with eggs of *G. batrachiensis* as commonly as tadpoles of *R. blairi* and *R. catesbeiana*. Alternatively, not all amphibian species may be equally susceptible to infections by this nematode as has been shown for other amphibian nematode and trematode species (Bolek and Janovy, 2007a, 2007b, 2008; Bolek at al., 2009, 2010; Johnson and Hartson, 2009; Langford and Janovy, 2009).

In contrast to hylid tadpoles, no reports exist for pinworm infections in tadpoles of pelobatid anurans or salamander larvae, and we never observed pinworm infections in tadpoles of *S. bombifrons* or larvae of *A. mavortium* (Muzzall and Schinderle,

TABLE IV. Morphological characters (reported as an average ± 1 SD) of adult male *Gyrinicola batrachiensis* from tadpole of 3 species of anurans collected from 2 locations in Nebraska.

	Host				
	*Rana catesbeiana**	*Rana pipiens*†	*Bufo woodhousii*†	Statistic	*P*
N	76	37	6		
Total length (mm)	1.14 ± 0.35	1.44 ± 0.45	1.21 ± 0.20	$H = 10.748$	=0.0046
Maximum width	130.66 ± 41.02	159.1 ± 59.18	88.33 ± 29.27	$H = 13.402$	=0.0012
Esophageal length	214.36 ± 56.63	219.64 ± 61.44	153.33 ± 12.11	$H = 6.771$	=0.0339
Pharyngeal bulb length	55.77 ± 10.47	57.66 ± 11.04	50.00 ± 6.32	$F = 1.454$	=0.2379
Pharyngeal bulb width	55.64 ± 10.86	57.65 ± 12.87	46.67 ± 12.11	$F = 2.346$	=0.1002
Nerve ring‡	115.40 ± 36.00	122.46 ± 37.15	110 ± 22.80	$F = 0.596$	=0.5528
Excretory pore‡	367.94 ± 219.65	370.55 ± 230.58	278.33 ± 143.17	$F = 1.869$	=0.1559
Spicule length	43.57 ± 8.39	47.45 ± 9.41	39.17 ± 4.38	$H = 10.227$	=0.006
Tail length	247.85 ± 79.82	280.42 ± 82.73	108.33 ± 29.94	$H = 18.899$	<0.0001

* Nevens Pond.
† Cedar Creek.
‡ Distance from anterior end.

TABLE V. *P* values for pair comparisons of morphological features of adult female *Gyrinicola batrachiensis* from various anuran tadpole hosts and locations collected from Nebraska. Bwb = *Bufo woodhousii* from Beckius Pond; Bwcc = *Bufo woodhousii* from Cedar Creek; Rc = *Rana catesbeiana*; Rp = *Rana pipiens*.

	Maximum width	Esophageal length	Pharyngeal bulb width	Nerve ring	Excretory pore	Tail length	Egg width
Rc, Bwcc	0.0107	0.0001	0.0022	0.0301	0.0251	0.144	0.5826
Rc, Bwb	0.0033	0.4191	0.0002	0.7551	0.5762	0.0063	0.016
Rc, Rp	0.0027	0.1302	0.0001	0.0002	0.3205	0.01	0.1433
Rp, Bwcc	0.2065	0.3878	0.1282	0.0576	0.5785	0.0004	0.5705
Rp, Bwb	0.1476	0.0001	0.8497	0.9999	0.0354	0.2429	0.0004
Bwb, Bwcc	0.6472	0.0022	0.6472	0.1134	0.0137	0.2061	0.0088

1992; Pryor and Greiner, 2004; Lannoo, 2005). Larvae of barred tiger salamanders are strictly carnivorous and take 2–5 mo to metamorphose, whereas tadpoles of the pelobatid *S. bombifrons* have a very short developmental period of 14–21 days, and are known to become cannibalistic in some populations (Petranka, 1998; Lannoo, 2005). Importantly, in our study, all 4 species of nematode-infected tadpoles are partially, or completely, herbivorous, supporting the idea that diet and the specialized digestive system of herbivorous tadpoles are important in the establishment of *G. batrachiensis* in larval amphibians (McDiarmid and Altig, 1999; Pryor and Greiner, 2004). In our study, larvae of *A. mavortium* were collected from Nevens Pond where American bullfrog tadpoles were infected with relatively high prevalence (56%) and mean abundance (6.8 ± 6.1) of *G. batrachiensis*, suggesting that salamander larvae had the opportunity of coming in contact with *G. batrachiensis* eggs but that they were resistant to infections with this pinworm. As in our study, Pryor and Greiner (2004) examined tadpoles of numerous amphibian species from Florida and noted that pelobatid tadpoles of *Scaphiopus holbrooki*, which are also known to be cannibalistic in some populations, were void of *G. batrachiensis* infections. However, they suggested that the extremely short developmental periods of 14 days in *S. holbrooki* tadpoles may prevent development and reproduction of *G. batrachiensis*, which has an estimated 9–19-day developmental period (Adamson, 1981a). Alternatively, they indicated that the highly carnivorous habits, characteristically short gastrointestinal tracts, or ephemeral pond habitats of *S. holbrooki* tadpoles might explain the absence of these nematodes in these amphibians. However, in our study, tadpoles of *S. bombifrons* were collected from a small roadside puddle that dried up within 3–4 wk of being formed, and no tadpoles of other anuran species occurred at this location. Thus, it is unclear whether eggs of *G. batrachiensis* were present for exposure to tadpoles of *S. bombifrons*, or whether tadpoles of pelobatids are resistant to infections with this nematode. Accordingly, until laboratory host specificity studies are conducted, it is unclear whether developmental period, carnivorous diet, shorter gut, ephemeral habitat, or combinations of these factors, play a role in the absence of *G. batrachiensis* from pelobatid tadpoles and salamander larvae.

TABLE VI. *P* values for pair comparisons of morphological features of adult male *Gyrinicola batrachiensis* from various anuran tadpole hosts and locations collected from Nebraska. Bwcc = *Bufo woodhousii* from Cedar Creek; Rc = *Rana catesbeiana*; Rp = *Rana pipiens*.

	Total length	Maximum width	Esophageal length	Spicule length	Tail length
Rc, Rp	0.0027	0.0005	0.9999	0.1222	0.1869
Rc, Bwcc	0.5812	0.0538	0.0031	0.4082	0.0006
Rp, Bwcc	0.3664	0.052	0.0054	0.0836	0.0004

Gyrinicola batrachiensis observed in all ranid tadpoles and *B. woodhousii* tadpoles from where bufonids were the only anuran species present, confirmed to the didelphic haplodiploidy and monodelphic parthenogenetic reproductive strategies, respectively (Adamson, 1981a; Table II). However, tadpoles of *B. woodhousii* that co-occurred with tadpoles of *R. pipiens* at Cedar Creek were inconsistent with these predictions and had both male and didelphic female nematodes (Table II). More importantly, female nematodes in tadpoles of *R. pipiens* and *B. woodhousii* from Cedar Creek were morphologically more similar to each other than to female nematodes from other anuran species, locations, or both (Table V), suggesting that tadpoles of these 2 anuran species shared a single population or reproductive strain of *G. batrachiensis*. However, although didelphic, female nematodes from tadpoles of *B. woodhousii* from Cedar Creek only produced thick-shelled eggs, whereas nematodes in tadpoles from *R. pipiens* from this location had both thick-shelled and thin-shelled eggs in their uteri. The low mean intensities (1.6 ± 0.7) of *G. batrachiensis* in tadpoles of *B. woodhousii* compared with the high mean intensities (14.9 ± 23.8) of this nematode in tadpoles of *R. pipiens* from Cedar Creek indicate that autoinfections were not occurring in tadpoles of *B. woodhousii* but that they were common in tadpoles of *R. pipiens*. These observations are important because laboratory infections of tadpoles of *R. clamitans* by Adamson (1981b) indicate that thin-shelled eggs are produced by female worms within 18–40 days of infection, suggesting that there was enough time for thin-shelled eggs to develop in female *G. batrachiensis* infecting tadpoles of *B. woodhousii* from Cedar Creek. Together, these data suggest that when populations of *G. batrachiensis* are shared among amphibian species that differ in developmental period, nematodes have an intermediate reproductive strategy in tadpoles with short developmental periods. These data suggest that strains of *G. batrachiensis* can alter their reproductive strategies based on host use, indicating reproductive plasticity in at least haplodiploid strains of these pinworms as has been reported in parasitic nematodes (Babayan et al., 2010). Clearly, cross-infection studies describing the development and reproduction of *G. batrachiensis* among tadpoles of amphibian species that differ in developmental period are needed to improve our understanding of the variation in reproductive strategies of these oxyurid nematodes.

Other studies by Pryor and Greiner (2004) and Adamson (1981d) have reported male nematodes, didelphic female nematodes, or both in tadpoles with short developmental periods (*Hyla*

femoralis and *Hyla versicolor*), whereas Adamson (1981c) reported both strains of haplodiploid and parthenogenetic females from the same individual in tadpoles with long developmental periods (*R. clamitans* and *R. pipiens*), indicating that tadpoles of different amphibian species share *G. batrachiensis* strains in nature. Unfortunately, none of those studies indicated whether tadpoles of other amphibian species infected with different strains of *G. batrachiensis* were present at these locations, or whether didelphic female nematodes in tadpoles of *H. femoralis* and *H. versicolor* produced autoinfective eggs. Our observations of low intensities and the absence of thin-shelled autoinfective eggs in didelphic female *G. batrachiensis* from tadpoles of *B. woodhousii* from Cedar Creek and the presence of thick-shelled eggs in gravid female worms suggest that *G. batrachiensis* is incapable of producing autoinfective eggs in tadpoles of *B. woodhousii*. Importantly, the 2 other studies that examined bufonid tadpoles for *G. batrachiensis* infections also indicated that *G. batrachiensis* in tadpoles of *B. americanus* and *B. terrestris* only produce thick-shelled eggs (Adamson, 1981a; Pryor and Greiner, 2004). These data suggest that tadpoles with short development, such as bufonids, can select for strains of *G. batrachiensis* that only produce thick-shelled eggs. Alternatively, nematodes may alter their reproductive strategy as a form of ''bet-hedging'' in different hosts as has been reported in other helminths (Thomas and Poulin, 2003). What is needed now are cross-transmission studies in different genera and species of amphibians with short developmental periods, such as bufonids and hylids, to determine whether tadpole developmental period or other amphibian genus-specific factors, species-specific factors, or both play a role in the reproductive strategy of this nematode. In addition, future experiments that cross male and female *G. batrachiensis* from different pinworm strains would also be informative in gaining a better understanding of the role of genetics for these alternative reproductive strategies. Such cross-infection studies should provide baseline data that will allow future testing of hypotheses into what host factors or reproductive strategies of nematodes selected for parthenogenetic strains of *G. batrachiensis*.

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