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NEW HOST AND DISTRIBUTION RECORDS FOR *APLECTANA HAMATOSPICULA* (ASCARIDIDA: COSMOCERCIDAE) IN *GASTROPHRYNE OLIVACEA* (ANURA: MICROHYLIDAE) FROM THE GREAT PLAINS U.S.A.

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ABSTRACT: *Aplectana hamatospicula* is a nematode that parasitizes the large intestine of anurans, and has been reported from bufonids, eleutherodactylids, hylids, microhylids, and ranids from North and Central America. *Aplectana hamatospicula* was first described, over 70 yr ago, from *Hyla eximia* and *Bufo peltocephalus* from Mexico and Cuba, respectively, and reported from *Gastrophryne carolinensis* from Florida. Since then there have been no reports of this nematode in North America north of Mexico. The life cycle of *A. hamatospicula* is not known, and there is limited information on *Aplectana* spp. from North America. During 2010–2011, we collected 351 anurans of 8 species from 4 locations in Stillwater, Payne County, Oklahoma, U.S.A., and examined them for the presence of *Aplectana* spp. Of the 8 species of anurans surveyed, *A. hamatospicula* infected only the Great Plains narrowmouth toad (*Gastrophryne olivacea*). The prevalence of *A. hamatospicula* was 85.7% (12/14) with a mean abundance of 28.4 + 24.0 and a mean intensity of 33.1 + 22.8 worms per infected toad. We provide new morphological measurements for male and female *A. hamatospicula* along with new locality records for this nematode in North America.

Although anurans are commonly surveyed for nematode parasites, few studies report *Aplectana* spp. from these hosts in North America. As a result, we know very little about the host specificity and distribution of cosmocercid nematodes in the genus *Aplectana*, including *Aplectana hamatospicula* Walton, 1940, a nematode reported to parasitize the large intestine of bufonids, eleutherodactylids and ranids in Cuba, hylids in Mexico, and the microhylid *Gastrophryne carolinensis* from Florida, U.S.A. (Walton, 1940; Barus, 1973; Martinez et al., 1982; Coy and Ventosa, 1984). *Aplectana hamatospicula* was first described, over 70 yr ago, from *Hyla eximia* and *Bufo peltocephalus* from Mexico and Cuba, respectively, and reported from *G. carolinensis* from Florida. Little is known of the life history of *Aplectana* spp. (Walton, 1940, 1941; Barus, 1973; Chabaud, 2009). As a result host specificity of these nematodes must be inferred from field surveys. The only information on a partial life cycle of any *Aplectana* spp. was provided by Chabaud and Brygoo (1958a, 1958b) who found that infective juveniles of *Aplectna courdurieri* failed to penetrate when placed in water on the skin of the semi-aquatic African mascarene grass frog, *Rana mascareniensis*. However, juvenile nematodes were ingested by, and developed in tadpoles of this ranid, and a few third stage juveniles left the intestine and were found in various tissues such as the liver and heart of exposed tadpoles. The authors hypothesized that worms acquired by tadpoles could persist through anuran metamorphosis, or adult frogs could acquire infections when they fed on infected tadpoles. However, the authors did not collect any field data to corroborate their laboratory infections. Since that time a number of *Aplectana* spp. have been described from terrestrial amphibians and lizards that do not feed on tadpoles, suggesting that some *Aplectana* spp. may have terrestrial life cycles as do other amphibian and reptile cosmocercid nematodes (Anderson, 2000; Bursey et al., 2006). In this study, we provide new host and geographical distribution information for *A. hamatospicula* along with new morphological measurements for male and female *A. hamatospicula*.

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MATERIALS AND METHODS

Description of field site and anuran collections

During 2010–2011 we collected 351 anurans of 8 species representing 4 families from 4 locations in Stillwater, Payne County, Oklahoma, U.S.A., and examined them for the presence of *Aplectana* spp. (see Table I). The locations included a shallow depression in a field, subsequently referred to as shallow depression (36°7.01′N, 97°3.00′W); Chapel Ridge Apartment complex and its surrounding area, referred to as Apartment Pond (36°8.337′N, 97°2.922′W); a depression in the lawn directly east of Boomer Lake (36°8.01′N, 97°3.01′W) referred to as Boomer Lake depression; and Teal Ridge, a non-irrigated, restored, semi-permanent wetland (36°6.050′N, 97°4.790′W).

Anurans were located with a spotlight by walking around the periphery of the ponds and depressions, and an attempt was made to collect as many anurans as possible regardless of species. However, because of their secretive nature all *Gastrophryne olivacea* were located by listening for mating calls. All anurans were captured by hand, placed in moist cotton bags, transported to the laboratory, double pithed, and necropsied within 48 hr of capture. Snout-vent length (SVL) and wet weight (WW) were recorded for each individual anuran to the nearest 1.0 mm and 0.01 g, respectively. At necropsy, the mouth, skin, digestive tract, limb and body wall musculature, body cavity, and internal organs were examined for all helminths. Each organ was placed individually in a petri dish and examined under a stereomicroscope. The body cavity of each anuran was then rinsed with tap water into a petri dish and the contents examined. Sex was determined for all individuals by gonadal inspection during necropsy. Nematodes were fixed in 70% ethanol and cleared in glycerol, according to Pritchard and Kruse (1982). All anuran nomenclature is according to Hillis (2007) and Pauly et al. (2009).

Morphological characteristics for worm identification and amphibian infection parameters

Prevalence, mean intensity, and mean abundance are reported according to Bush et al. (1997). All values are reported as the mean + 1 standard deviation (SD). Nematodes were identified based on descriptions by Walton (1940, 1941) and Barus (1973). Voucher specimens have been deposited in the H.W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accession numbers HWML 67159, male *Aplectana hamatospicula*; and HWML 67160, female *A. hamatospicula*.

Measurements on 10 individual male and female *A. hamatospicula* from representative anurans from each location were taken using a calibrated micrometer on an Olympus BX-51 upright research microscope configured for brightfield and differential interference contrast microscopy with plain fluorite objectives, and digital photographs were obtained using an Olympus 5 megapixel digital camera. The following measurements were recorded for male nematodes: total length. greatest width, length of esophagus, length of pharyngeal bulb, greatest width of pharyngeal bulb, distance of excretory pore from anterior end, distance of nerve ring from

TABLE I. Locations, species, and numbers of anurans (separated by year) collected in Stillwater, Payne County, Oklahoma, U.S.A., and examined for *Aplectana hamatospicula*.

Location	Species	No. anurans in 2010	No. anurans in 2011	Total
Teal Ridge	*Acris crepitans*	21	24	45
	Bufo americanus	2	15	17
	Bufo woodhousii	3	5	8
	Gastrophryne olivacea	0	1	1
	Hyla versicolor	4	45	49
	Rana catesbeiana	34	3	37
	Rana sphenocephala	24	74	98
Apartment Pond	*B. americanus*	32	0	32
	B. woodhousii	3	0	3
	G. olivacea	4	1	5
	R. catesbeiana	27	9	36
	R. sphenocephala	1	0	1
Shallow depression	*A. crepitans*	3	0	3
	B. americanus	4	0	4
	B. woodhousii	2	0	2
	G. olivacea	6	0	6
	H. versicolor	2	0	2
	Pseudacris clarkii	1	0	1
Boomer Lake depression	*G. olivacea*	2	0	2

anterior end, distance of posterior end of tail from the cloaca, spicule length, and gubernaculum length. The following measurements were recorded for female nematodes: total length, greatest width, length of esophagus, the length of pharyngeal bulb, greatest width of pharyngeal bulb, distance of excretory pore from anterior end, distance of nerve ring from anterior end, distance of vulva from anterior end, distance of posterior end of tail from the anus, greatest width at vulva, and egg length and egg width. All measurements are reported as a range in microns unless otherwise noted.

RESULTS

Of the 8 anuran species examined (Table I) only the Great Plains narrowmouth toad, *G. olivacea,* was infected with *A. hamatospicula* at each location A total of 12 male and 2 female adult *G. olivacea* (mean wet weight = 2.2 + 0.5 g [1.6–3.2]. mean snout-vent length = 3.1 + 0.2 cm [2.7–3.5]) were collected and including 6 individuals from shallow depression, 5 individuals from Apartment Pond, 2 individuals from Boomer Lake depression, and 1 individual from Teal Ridge. The prevalence of *A. hamatospicula* was 85.7 % (12/14) with a mean abundance of 28.4 + 24.0 and a mean intensity 33.1 + 22.8 worms per infected frog. Morphological measurements for male and female worms are provided below.

Aplectana hamatospicula Walton, 1940

General: Cuticle of body with fine transverse striations. Lateral alae present. Somatic papillae present over body surface, in 2 subventral and 2 subdorsal rows. Cephalic end with 3 small lips. Anterior extremity of esophagus in form of 3 blunt projections covered with thick ring of cuticle. Esophagus divided into short anterior pharyngeal portion, elongate corpus, short and narrow isthmus, and large valved bulb.

Males: Total length 1.92–2.15 mm. Greatest width 81.68–112.31. Esophagus total length 326.72–398.19; pharyngeal bulb length 61.26–91.89 and width 56.16–81.68. Nerve ring 183.78–224.64 and excretory pore distance from anterior end 257.67–357.35. Tail length from cloaca 102.10–173.57. Caudal papillae distributed as follows: 1 median and 4 pairs precloacal and 7 pairs postcloacal. Spicules equal 234.36–244.44 long. The spicules are hamate at their distal ends and have the tips covered by a cuticularized cap-like structures (Fig. 1). However, this cuticularized cap-like structure disintegrated sometime within 4 wk when nematodes were left in glycerol. Gubernaculum well sclerotized; 32.04–69.42 long.

Females: Total length 2.25–3.40 mm. Greatest width 102.10–173.57. Width at vulva 61.26–81.68. Esophagus total length 357.35–418.61; pharyngeal bulb length 71.47–102.10 and width 71.47–102.10. Nerve ring 183.88–245.04, excretory pore 285.88–367.56, and vulva 2.22–3.35 mm from anterior end. Tail length from anus 132.73–193.99, conical and slender. Amphidelphic, ovary of anterior uterus terminating anterior to uterus, ovary of posterior uterus terminating just anterior to vulva. Eggs 61.26–81.68 long by 30.63–51.05 wide; containing developing juveniles.

DISCUSSION

Walton (1940, 1941) described *A. hamatospicula* from *H. eximia* and *B. peltocephalus* from Mexico and Cuba, respectively, and reported this species from *G. carolinensis* collected from Florida, but did not provide any morphological characteristics from this host. Since its description no other reports of *A. hamatospicula* exist for the United States. In a later study, Barus (1973) provided additional morphological characteristics for *A. hamatospicula* from *B. peltocephalus* and *Bufo taladai* collected in Cuba. Finally, Coy and Ventosa (1984) reported this nematode from 14 anuran species (*B. peltocephalus*, *Bufo longinasus, Bufo empusus*, *B. taladai*, *Eleutherodactylus albipes*, *Eleutherodactylus cuneatus*, *Eleutherodactylus dimidiatus*, *Eleutherodactylus gundlachi*, *Eleutherodactylus klinikow-*

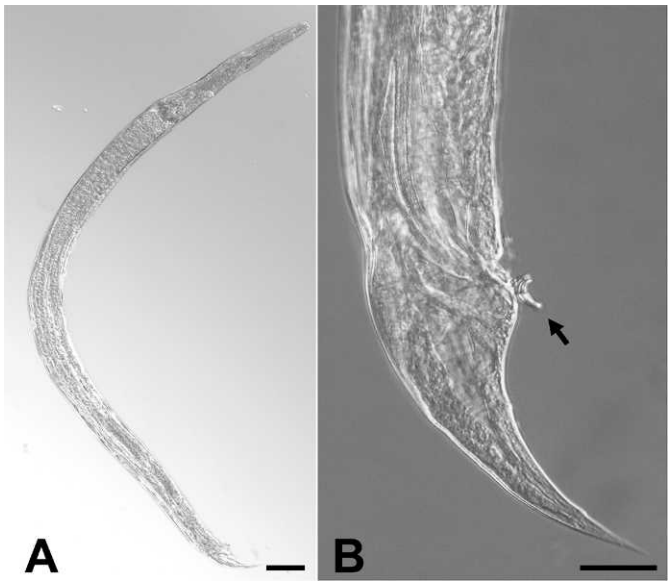

FIGURE 1. Entire worm (**A**) and posterior end (**B**) of a male *Aplectana hamatospicula* showing the distinct spicule cap (black arrow). Scale bars = 100 μm for A and 50 μm for B.

TABLE II. Morphological characteristics for male *Aplectana hamatospicula* collected from Cuba, Mexico, and the United States.

	Oklahoma, U.S.A. (present study)	Cuba and Mexico (Walton, 1940)	Cuba (Barus, 1973)
Host(s)	*G. olivacea*	*Bufo peltocephalus* and *Hyla eximia*	*B. peltocephalus* and *Bufo taladai*
No. worms measured	10	NG†	NG†
Total length (mm)	1.92–2.15 mm	2.09–3.05 mm	3.43–3.77 mm
Greatest width	82–112	109–122	140–200
Esophageal length	327–398	435–480	390–450
Pharyngeal bulb length	61–92	80–90	87–100
Pharyngeal bulb width	56–82	80–90	NG†
Nerve ring*	184–225	236–245	240–260
Excretory pore*	258–357	461–470	450–480
Spicule length	234–244	236–245	220–290
Gubernaculum length	32–69	NG†	65–73
Tail length	102–173	127–145	150–180

* Distance from anterior end.
† NG = not given.

skii, *Eleutherodactylus sierramaestrae*, *Eleutherodactylus symingtoni*, *Eleutherodactylus zeus*, *Osteopilus septentrionalis*, and *Rana catesbeiana*) from Cuba but they did not report any morphological characteristics for *A. hamatospicula* from these hosts.

Morphological comparisons of male and female *A. hamatospicula*, from our study, to measurements provided for male and female *A. hamatospicula* by Walton (1940) and Barus (1973), indicate that worms recovered from *G. olivacea* are more similar to worms recovered from *H. eximia* and *B. peltocephalus* by Walton (1940) than the worms recovered from the 2 bufonid species by Barus (1973; see Tables II and III). Because Walton (1940, 1941) and Barus (1973) did not provide any information on how many worms they measured and from what host(s) species those measurements were reported from, it is unclear if host-induced morphological variation occurs in this species of nematode in hylid and bufonid anurans. However, our data clearly indicate that *A. hamatospicula* from *G. olivacea* collected in Oklahoma are morphologically distinct in some of their characteristics from *A. hamatospicula* collected from hylid and bufonid anurans from Mexico and Cuba by Walton (1940) and Barus (1973). A recent study by Rhoden and Bolek (2011) on the morphology of another amphibian nematode, *Gyrinicola batrachiensis*, indicates that host-induced morphological variation is a common phenomenon in other amphibian nematodes. These authors indicated that both host species and the locality from where *G. batrachiensis* was collected affected worm morphology. Although our specimens of *A. hamatospicula* were morphologically distinct in most of their morphological characteristics from the report of Barus (1973), the most significant distinguishing characteristics for species identification in the genus *Aplectana* are the number of caudal papillae and spicule morphology including the cuticularized cap-like structures covering the distal ends of spicules (see Fig. 1; Bursey et al., 2006). Our specimens of *A. hamatospicula* agreed with the numbers and position of caudal papillae and spicule morphology reported for males of this species by Walton (1940, 1941) and Barus (1973). Additionally, there was overlap in spicule size for male *A. hamatospiula* in our study and spicule size reported for males of *A. hamatospiula* by Walton (1940) and Barus (1973), suggesting that these morphological characteristics are more useful for species identification in this genus than are length and width measurements of typical nematode characteristics reported in Tables II and III. However,

TABLE III. Morphological characteristics for female *Aplectana hamatospicula* collected from Cuba, Mexico, and the United States.

	Oklahoma, U.S.A. (present study)	Cuba and Mexico (Walton, 1940)	Cuba (Barus, 1973)
Host(s)	*G. olivacea*	*B. peltocephalus* and *H. eximia*	*B. peltocephalus* and *B. taladai*
No. worms measured	10	NG†	NG†
Total length (mm)	2.25–3.40 mm	3.41–4.20 mm	3.58–4.21 mm
Greatest width	102–174	NG†	220–270
Width at vulva	61–82	145–185	NG†
Esophageal length	357–419	472–525	310–470
Pharyngeal bulb length	71–102	90–100	110–130
Pharyngeal bulb width	71–102	90–100	110–130
Nerve ring*	184–245	254–280	240–270
Excretory pore*	286–368	480–508	380–490
Vulva*	2.22–3.35 mm	2.90–3.40 mm	2.56–2.61 mm
Tail length	132–193	162–220	180–220
Egg length	61–82	85–109	100–110
Egg width	31–51	55–65	74–85

* Distance from anterior end.
† NG = not given.

our observation of the disintegration of the cap-like structures covering the distal ends of the spicules when *A. hamatospicula* is left in glycerol indicates that *Aplectana* spp. need to be examined for their spicule morphology quickly after being placed in glycerol. Taken together, our observations of *A. hamatospicula* indicate that it is critical for investigators who conduct surveys to report basic morphological characteristics for helminths reported in their studies and deposit voucher specimens from each host species infected with a particular helminth species. Only then will we be able to evaluate if host induced morphological variation occurs in other anuran nematodes.

In our study Great Plains narrowmouth toads, *G. olivacea*, were the only anurans infected with *A. hamatospicula* and represent a new host record and new locality record for this nematode. Great Plains narrowmouth toads are small bodied, fossorial anurans. They prefer to reside in dry open areas under rocks, and they are known to occupy spider and gopher burrows, spending a significant portion of their life beneath the ground (Collins, 1993). Because of their secretive life style, *G. olivacea* are difficult to collect and few studies exist on their helminth parasites, and our discovery of *A. hamatospicula* in this anuran probably represents the lack of survey data on this anuran species (Kuntz, 1941; Harwood, 1932; Freiburg, 1951; McAllister and Upton, 1987; Goldberg et al., 1998). In the current study *G. olivacea* was the most fossorial amphibian species found at all 4 collection sites, and *A. hamatospicula* appeared to be host specific to this anuran. In terms of amphibian species, 3 of the locations where infected *G. olivacea* were collected had multiple species of amphibians (see Table I). Despite this, *A. hamatospicula* was recovered only from *G. olivacea*, suggesting either *A. hamatospicula* is host specific to *G. olivacea* or *G. olivacea* frequents a specific type of microhabitat not frequented by other anuran species in our study. The latter is more likely due to reports of *A. hamatospicula* occurring in other anuran species from 4 families in Central America (Walton, 1940; Schwartz and Ogren, 1956; Barus, 1973; Coy and Ventosa, 1984). In fact, the most commonly reported host for *A. hamatospicula* is the Cuban *B. peltocephalus*, a fossorial toad which resides in small burrows or under large rocks, similar to the microhabitat preferred by *G. olivacea* (Walton, 1940; Schwartz and Ogren, 1956; Barus, 1973). This shared microhabitat of *G. olivacea* and *B. peltocephalus* suggests that these toads are more likely to encounter *A. hamatospicula* than other anuran species that are less fossorial. Goldberg et al. (1998) report that *Aplectana incerta* and *Aplectana itzocanensis* infected 8 and 11 fossorial anurans, respectively. This indicates that other species in the genus *Aplectana* are not host specific, further supporting the hypothesis that *A. hamatospicula* is not host specific to microhylids, but may be an amphibian nematode infecting more fossorial species of anurans. Clearly, controlled experimental host specificity studies along with molecular data on field-collected specimens are needed to test the hypothesis of compatibility or encounter filters in explaining the observed host specificity of *A. hamatospicula* in Oklahoma anurans (Euzet and Combes, 1980). Finally, further surveys of Great Plains narrowmouth toads and other anurans are needed to define the distribution and host range of *A. hamatospicula*.

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