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Helminths from the Hellbender, *Cryptobranchus alleganiensis* (Urodela: Cryptobranchidae), in Missouri, U.S.A.

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ABSTRACT: Two hellbenders, *Cryptobranchus alleganiensis* (Urodela: Cryptobranchidae), from Missouri, U.S.A., were examined for helminths. Three nematode species, *Falcaustra catesbeianae* Walton, 1929, *Urodelenema mackini* (Walton, 1941), and *Kamegainema cingula* (Linstow, 1902) and 1 proteocephalid cestode species were found. This is the first record of *Fa. catesbeianae* from the hellbender. *Kamegainema cingula*, normally a dermal nematode of cryptobranchids, is reported for the first time infecting the body cavity.

KEY WORDS: helminths, *Falcaustra catesbeianae*, *Urodelenema mackini*, *Kamegainema cingula*, proteocephalid cestode, hellbender, *Cryptobranchus alleganiensis*, U.S.A., Missouri, Nematoda, Urodela, Amphibia.

The hellbender, *Cryptobranchus alleganiensis* (Urodela: Cryptobranchidae), is a large salamander distributed in certain rivers of the eastern United States (Pough et al., 1998). Helminthological surveys of this amphibian have been carried out since the early twentieth century (La Rue, 1914; Krecker, 1915). However, our knowledge of the parasites of the hellbender remains incomplete. We recently examined 2 hellbenders and recovered 4 helminth species. Herein, we report the results, including a new host and a new infection site record.

MATERIALS AND METHODS

The hellbenders examined were originally collected in April 1987 from the Niangua River, Dallas County, Missouri, U.S.A., by R. F. Wilkinson, Southwest Missouri State University, Springfield, Missouri, U.S.A., for an experimental study of feeding mechanics (Elwood and Cundall, 1994). They were maintained for about 1 wk at Lehigh University, Bethlehem, Pennsylvania, U.S.A., in a flow-through system that had previously contained hellbenders from other sites and were then transported to Harvard University, Cambridge, Massachusetts, U.S.A., where they were housed together in a 400-liter aquarium for approximately 5 wk. Subsequently, hellbenders were housed individually in smaller aquaria for approximately 2 wk during physiological and behavioral recording sessions. When the hellbenders arrived from Missouri, they regurgitated bits of crayfish exoskeleton, but none of them ate for about 1 mo after they were transported to Harvard. After 1 mo they began to eat live baby mice and live goldfish a few weeks before they were killed. After the experiments the hellbenders were killed with tricaine methanesulfonate and fixed in 10% formalin.

A helminthological survey was conducted on viscera excised from fixed specimens transported to our laboratory. The visceral surface was carefully examined under a stereomicroscope for pathological changes associated with helminthic infection. Then, the alimentary canal was cut open and washed on a 0.07-mm-aperture sieve. The residue left on the sieve was transferred to a petri dish and examined for helminths under a stereomicroscope. The lungs were cut open and examined. Helminths were cleared in an alcohol-glycerol solution by evaporation, mounted on a glass slide with 50% glycerol solution, and observed under a Nikon Optiphot microscope equipped with a Nomarski interference contrast apparatus. Measurements (range values followed parenthetically by mean \pm SD) are given in micrometers unless otherwise stated. Specimens are deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. The hosts are deposited in the Department of Behavioral and Evolutionary Biosciences, Lehigh University, Bethlehem, Pennsylvania, U.S.A., with accession numbers LU 2377 and 2378.

The following specimens were also examined for comparison—*Falcaustra catesbeianae* Walton, 1929 ex *Rana catesbeiana* Shaw, 1802: USNPC 91246; *Kamegainema cingula* (Linstow, 1902) ex *Andrias japonicus* (Temminck, 1836): personal collection of T. Tochimoto, Himeji, Hyogo, Japan.

Cryptobranchus alleganiensis

Two individuals collected from the Niangua River, Dallas County, Missouri, U.S.A.: one male, snout-vent length (SVL) 29.5 cm; 1 female, SVL 30.5 cm.

Falcaustra catesbeianae Walton, 1929

(Nematoda: Kathlaniidae)

Description: General morphology almost identical with that described by Baker (1986).

Males: ($n = 11$.) Length 7.18–9.74 (8.04 \pm 0.87) mm, width in midbody 267–345 (300 \pm

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23). Pharynx 61–72 (65 ± 3) long; esophageal corpus 0.9–1.02 (0.95 ± 0.03) mm long by 70–77 (74 ± 3) wide near posterior end; isthmus 86–115 (102 ± 9) long by 93–99 (97 ± 2) wide; bulb 144–165 (155 ± 6) long by 141–156 (149 ± 6) wide. Nerve ring 275–323 (303 ± 16), excretory pore 0.88–1.06 (0.95 ± 0.05) mm, and deirids 0.75–0.92 (0.78 ± 0.05) mm from anterior extremity. Spicules 294–343 (314 ± 18) long. Gubernaculum 64–69 (67 ± 2) long. Pseudosucker 1.58–2.79 (1.93 ± 0.39) mm from posterior extremity. Tail 409–494 (451 ± 28) long.

Females: ($n = 11$.) Length 7.72–9.34 (8.3 ± 0.51) mm, width in midbody 275–307 (293 ± 13). Pharynx 64–70 (65.5 ± 2.4) long; esophageal corpus 0.91–1.05 (0.98 ± 0.04) mm long by 70–82 (77.6 ± 3.4) wide near posterior end; isthmus 96–112 (103 ± 5) long by 99–109 (104 ± 4) wide; bulb 147–170 (156 ± 8) long by 154–163 (158 ± 3) wide. Nerve ring 291–326 (307 ± 9), excretory pore 0.91–1.06 (0.96 ± 0.04) mm, and deirids 0.74–0.84 (0.8 ± 0.04) from anterior extremity. Vulva 4.94–5.98 (5.4 ± 0.34) mm (i.e., 62–67% [$65 \pm 1.7\%$] of worm length) from anterior extremity. Vagina 601–704 (655 ± 28) long. Tail 520–689 (620 ± 60) long. Eggs 77–102 (91.4 ± 5.6) by 61–74 (65.8 ± 2.5) ($n = 45$).

Host: *Cryptobranchus alleganiensis* (Daudin, 1802).

Site of infection: Rectum.

Intensity: Fifty-seven (25 males, 32 females); 7 (3 males, 4 larvae).

Locality: Niangua River, Dallas County, Missouri, U.S.A. (approximately 37°45'N; 92°54'W).

Date of collection: April 1987.

Specimens deposited: Eleven males and 11 females in USNPC 92351.

Remarks: *Falcaustra catesbeiana* is a common nematode of the bull frog, *R. catesbeiana*, but it also has been recorded from various amphibians of North America, including the lesser siren, *Siren lacertina* Linnaeus, 1766, and the grotto salamander, *Typhlotriton spelaeus* Stejneger, 1893 (Baker, 1987). This nematode is characterized by its short, robust spicules. In the original description of *Fa. catesbeiana*, Walton (1929) stated that the male “has a median double precloacal papilla,” giving a figure of a lateral view

of a male tail with 2 projections on the median precloacal papilla. Later, Reiber et al. (1940) reported that the male possesses “1 median pair of preanal papillae.” Such paired median precloacal papillae are quite unusual for *Falcaustra* species (cf. Bursey et al., 2000). Moreover, the worms studied by Walton (1929) and Reiber et al. (1940) were significantly smaller (males 3.5–4.48 mm long, females 4–6.16 mm). However, according to the redescription by Baker (1986), based on material collected from the type host *R. catesbeiana*, the precloacal median papilla is single, and the worm size is comparable with that of the worms recovered in our study. Although the male tail reported herein is significantly longer than that recorded by Baker (1986) (336–334 μm), the males in USNPC 91246 had tail length (423–437 μm in worms 8–8.9 mm long) similar to that of our samples. This is the first record of *Fa. catesbeiana* from *Cr. alleganiensis*.

***Urodelnema mackini*
(Walton, 1941) Baker, 1981**

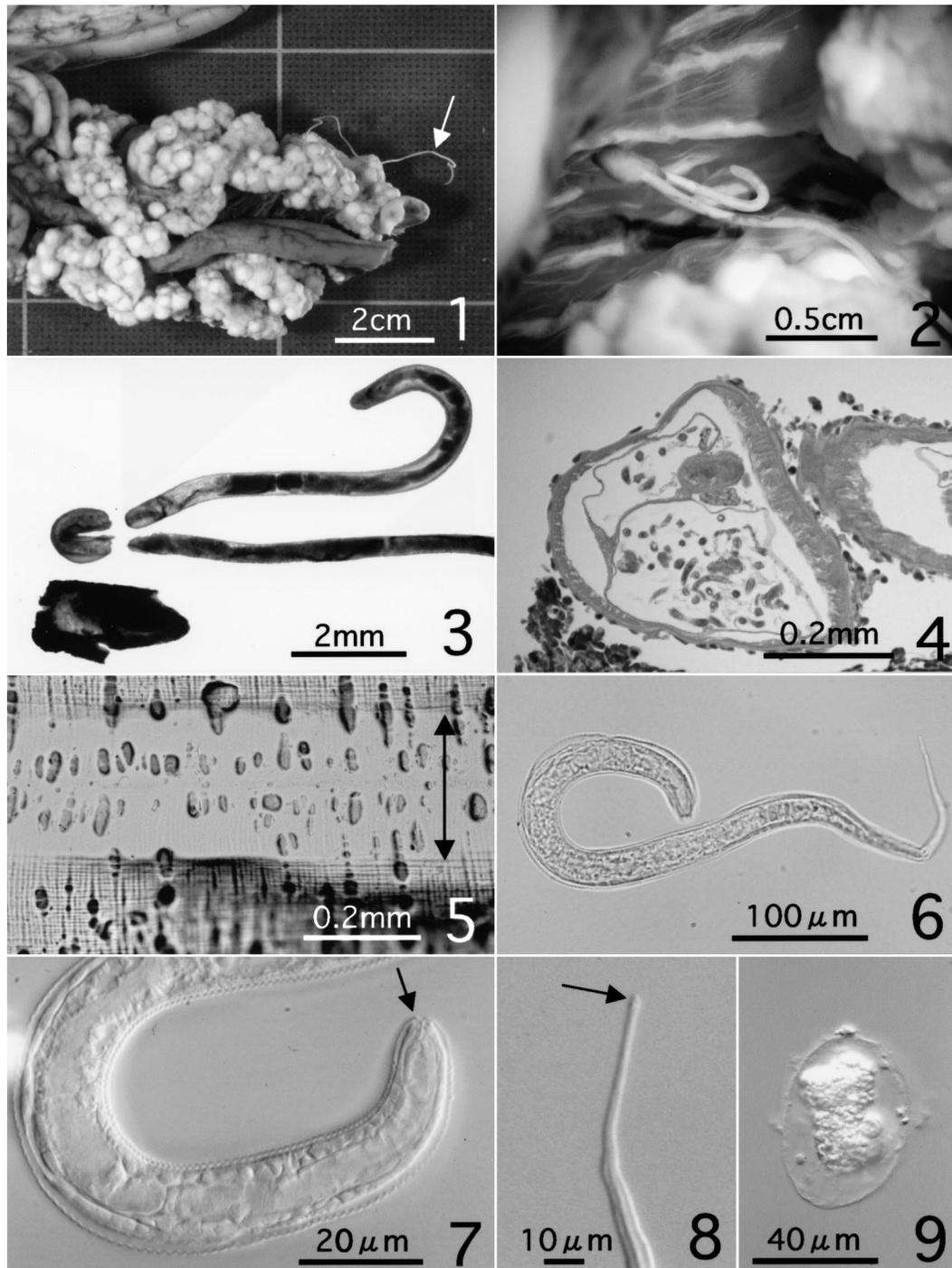
(Syn. *Zanclophorus cryptobranchi* Walton, 1930; *Spironoura mackini* Walton, 1941; *Falcaustra waltoni* Freitas et Lent, 1941; *Falcaustra mackini* (Walton, 1941) Chabaud et Golvan, 1957; *Cordonema mackini* (Walton, 1941) Baker, 1981)

(Nematoda: Kathliniidae)

Description: Morphology identical to that described by Baker (1981).

Males: ($n = 10$.) Length 6.61–7.34 (6.98 ± 0.30) mm, width in midbody 246–325 (277 ± 22). Pharynx 70–80 (75 ± 4) long; esophageal corpus 1.12–1.3 (1.2 ± 0.06) mm long by 58–64 (60 ± 3) wide in midlength; isthmus 143–173 (158 ± 9) long by 64–77 (71 ± 4) wide; bulb 166–192 (176 ± 7) long by 137–166 (154 ± 8) wide. Nerve ring 410–468 (434 ± 17), excretory pore 748–882 (809 ± 47), and deirids 623–813 (675 ± 57) from anterior extremity. Spicules 700–803 (762 ± 42) long. Gubernaculum 112–128 (121 ± 4) long. Pseudosucker 1.95–2.44 (2.18 ± 0.15) mm from posterior extremity. Tail 421–484 (450 ± 22) long.

Females: ($n = 10$.) Length 7.08–8.75 (7.61 ± 0.45) mm, width in midbody 234–392 (281 ± 55). Pharynx 67–80 (75 ± 4) long; esophageal corpus 1.13–1.36 (1.24 ± 0.07) mm long by 51–



Figures 1-9. Female of *Kamegainema cingula* (Linstow, 1902) found in the body cavity of the hellbender, *Cryptobranchus alleganiensis*, in U.S.A. **1.** Viscera of hellbender showing the worm (arrow). **2.** Anterior portion of the worm with cervical region embedded in a cellular mass. **3.** Anterior portion excised from the cellular mass. **4.** Cross-section of the cervical region showing numerous larvae in the uteri and host cells adhered on

64 (62 ± 4) wide in midlevel; isthmus 143–169 (154 ± 9) long by 67–80 (75 ± 4) wide; bulb 163–195 (185 ± 9) long by 154–186 (164 ± 11) wide. Nerve ring 413–502 (453 ± 30), excretory pore 779–934 (835 ± 44), and deirids 629–746 (688 ± 36) from anterior extremity. Vulva 4.44–5.67 (4.93 ± 0.31) mm (i.e., 62–66% [$65 \pm 1\%$] of worm length) from anterior extremity. Vagina 488–789 (635 ± 97). Tail 600–723 (655 ± 36) long. Eggs 83–99 (90 ± 5) by 64–74 (69 ± 3) ($n = 27$).

Host: *Cryptobranchus alleganiensis* (Daudin, 1802).

Site of infection: Rectum.

Intensity: Approximately 350 (155 males, 190 females).

Locality: Niangua River, Dallas County, Missouri, U.S.A. (approximately 37°45'N; 92°54'W).

Date of collection: April 1987.

Specimens deposited: Ten males and 10 females in USNPC 92352.

Remarks: *Urodelnema mackini* was first described as *Z. cryptobranchi* by Walton (1930), along with *Spironoura cryptobranchi* Walton, 1930, both collected from *Cr. alleganiensis* (Walton, 1930). However, when subsequently transferred to *Spironoura*, it was renamed *Sp. mackini* to resolve the secondary homonymy (Walton, 1941). Forty years later, a new genus, *Cordonema* Baker, 1981, was erected for the 3 kathlaniids parasitic in the hellbender (Baker, 1981). However, this generic name was already taken by *Cordonema* Schmidt et Kuntz, 1972 in Acuariidae (Nematoda), and ultimately *Urodelnema* Baker, 1981 was proposed (see Baker, 1987).

***Kamegainema cingula* (Linstow, 1902)
Hasegawa, Doi, Araki, and Miyata, 2000
(Figs. 1–9)**

(Syn. *Filaria cingula* Linstow, 1902)

(Nematoda: Dracunculidae)

One female was found in the body cavity (Fig. 1). The posterior end was lost in dissection. The cervical region of the worm was enclosed in an ellipsoidal dark mass, in which the worm body was folded and constricted 2 times (Figs. 2, 3). This ellipsoidal mass was connected to the membranous tissue around the kidney by 2 thin fibers.

Description: Body filiform, total length of fragments 78 mm, width almost uniform (0.5 mm). Anterior portion covered with infiltrated cells (Fig. 4). Cuticle with transverse embossment of irregular length, often globular. On cuticular surface over lateral cords, embossment forming 2 irregular longitudinal rows (Fig. 5). Esophagus divided into anterior muscular and posterior glandular portions. Intestine atrophied. Uteri filled with larvae and eggs (Figs. 4, 6–9). Uterine larvae 216–278 (257 ± 21) long, 13–16 (14 ± 1) wide ($n = 17$). Cephalic extremity with rudimentary tooth dorsally (Fig. 7). Esophagus and tail occupying 26% and 31% of worm length, respectively. Tail slender and slightly expanded distally, forming blunt tip (Fig. 8). Phasmids with vesicles and pores of moderate size, located slightly posterior to anus. Uterine eggs with thin fragile shell, 51–66 (59 ± 4.8) by 35–46 (40.8 ± 3.4) ($n = 10$), containing embryos at various developmental stages (Fig. 9).

Host: *Cryptobranchus alleganiensis* (Daudin, 1802).

Site of infection: Body cavity.

Intensity: One female.

Locality: Niangua River, Dallas County, Missouri, U.S.A. (approximately 37°45'N; 92°54'W).

Date of collection: April 1987.

Specimens deposited: One female in USNPC 92354.

Remarks: *Kamegainema cingula* was first described as *Fi. cingula* from the Japanese giant salamander, *A. japonicus*, reared in Hamburg, Germany (Linstow, 1902). Recently, a new ge-

←

the cuticular surface. **5.** Cuticular surface showing bosses grossly arranged in 2 longitudinal rows on the lateral field of which width is indicated by an up–down arrow. **6.** Uterine larva. **7.** Anterior portion of the uterine larva, showing a cephalic tooth (arrow). **8.** Posterior end of the uterine larva, showing slightly inflated tip (arrow). **9.** Uterine egg.

nus, *Kamegainema* Hasegawa, Doy, Araki, and Miyata, 2000, was proposed for it (Hasegawa et al., 2000; but see also Hasegawa et al., 2002 for a nomenclatural note). This nematode has been collected only once in Ohio, U.S.A., from *Cr. alleganiensis* (Krecker, 1915). *Kamegainema cingula* was reported previously only from the skin, and this is the first report of this nematode from the body cavity. The morphology of the present female was generally consistent with previous descriptions, but there are several discrepancies. The distribution pattern of the bosses in the present worm differs somewhat from that described by Krecker (1915), who found 4 longitudinal rows of "papillae" over the lateral field. The Japanese examples also differ in that their bosses are not arranged in 2 longitudinal rows in the lateral field (Hasegawa et al., 2000).

The present uterine larvae have a less developed cephalic tooth and phasmidial pores and a less pointed tail tip than do those in *K. cingula* from *A. japonicus* (cf. Hasegawa et al., 2000). Presumably, these discrepancies in the larval morphology are due to their younger condition because the uterine larvae of *K. cingula* were much longer in females from *Cr. alleganiensis* (330 μm long; Krecker, 1915) and *A. japonicus* (334–455 μm long; Hasegawa et al., 2000).

Proteocephalidae gen. sp.

(Cestoda: Proteocephalidae)

Description: Fragments of 3 strobilae. Scolex trapezoidal with 4 large suckers and minute sucker-like apical organ. Genital organs not developed.

Host: *Cryptobranchus alleganiensis* (Daudin, 1802).

Site of infection: Small intestine.

Intensity: Three.

Locality: Niangua River, Dallas County, Missouri, U.S.A. (approximately 37°45'N; 92°54'W).

Date of collection: April 1987.

Specimens deposited: Three strobilae with scolexes in USNPC 92355.

Remarks: Although the present strobilae were immature, they are presumably *Ophiotaenia cryptobranchi* La Rue, 1914, which is reported from *Cr. alleganiensis* and other salamanders of North America (cf. La Rue, 1914; Dyer and Brandon, 1973).

DISCUSSION

The family Cryptobranchidae contains only 3 living members: *Cr. alleganiensis* in the U.S.A., *A. japonicus* in Japan, and the Chinese giant salamander, *A. davidianus* (Blanchard, 1871), in China (Pough et al., 1998). *Urodelnema* is specific to the hellbender. It is closely related to *Falcaustra*: the only difference is the presence of cordons in *Urodelnema*, which may be advantageous for adhering to the rectal wall of the hellbender. Baker (1981) postulated that *Urodelnema* evolved directly from *Falcaustra* within the cryptobranchid salamanders. Hence, a mixed infection with *Urodelnema* and *Falcaustra* in a hellbender is of interest. The presence of *Fa. catesbeianae* is not surprising because it has a wide host range, including ranid and hylid anurans, and sirenid and plethodontid urodelans in North America (Baker, 1987). Interestingly, *Urodelnema* and *Falcaustra* have not been reported from *A. japonicus*, but another specific kathlaniid, *Megalobatrachonema nipponicum* Yamaguti, 1941, the type species of the genus, is known from this host (Yamaguti, 1941).

The presence of the female of *K. cingula*, a dermal parasite, in the body cavity is of special interest. This female seems to have not attained a fully gravid state because the uterine larvae are incompletely developed. It is suggested that females of this nematode first inhabit the body cavity and then migrate to the skin after maturation to liberate larvae into the water (cf. Anderson, 2000). The life history of *K. cingula* has not been elucidated, but presumably freshwater cyclopoid copepods and fish act as intermediate and paratenic hosts, respectively (cf. Anderson, 2000).

Kamegainema cingula is the only species that is known to parasitize both *A. japonicus* and *Cr. alleganiensis*. The same nematode species is unusual in the amphibians of Asia and North America. The differences in the arrangement of cuticular bosses suggests that some extent of speciation has occurred in *Kamegainema* of both regions. Unfortunately, there are few specimens of *K. cingula* from the hellbenders available for study. Moreover, no male of *K. cingula* is known. Further study is necessary to elucidate the biology of this dracunculid.

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Obituary Notice

A. MORGAN GOLDEN

1920–2002

Elected to membership March 18, 1953

Recording Secretary 1973–1974	Brayton H. Ransom Memorial Trust Fund
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President 1976	Chairman 1979–1982
Editorial Board Member 1984–1999	President 1983–1994
Anniversary Award Recipient 1985	Trustee 1995–2002
Elected to Life Membership 1989	