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Research Note

Metacercariae of *Clinostomum attenuatum* in *Ambystoma tigrinum mavortium*, *Bufo cognatus* and *Spea multiplicata* from west Texas

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Abstract

Tissues from barred tiger salamanders (*Ambystoma tigrinum mavortium*), Great Plains toads (*Bufo cognatus*) and New Mexico spadefoots (*Spea multiplicata*) collected from 16 playa wetlands in Texas during 1999 and 2000 were examined by light microscopy. Digenean cysts were primarily distributed subcutaneously throughout the specimens and occasionally coelomic invasion was noted. The parasites within the cysts were 1.5–2 mm in diameter, with a thin (*c.* 10 μm wide) eosinophilic-staining tegument, two suckers (oral and ventral), posteriorly located primordial genitalia and paired digestive caeca. These digeneans were identified as the metacercariae of *Clinostomum attenuatum*. This is the first record of *Clinostomum attenuatum* in these amphibian species.

Twenty-one amphibians (eight barred tiger salamanders (*Ambystoma tigrinum mavortium*), two Great Plains toads (*Bufo cognatus*), and 11 New Mexico spadefoots (*Spea multiplicata*) were collected from 16 playa (Smith, 2003) wetlands in Texas during 1999 and 2000. The amphibians were euthanized (Texas Tech University Animal Care and Use Committee permit number 99843), placed in 10% buffered formalin and submitted to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory (UGA Tifton VDIL) for full necropsies to confirm and characterize suspected parasitism. Gross observations were made on fixed specimens.

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Digenean cysts were observed in both Great Plains toads, seven of the New Mexico spadefoots and five of the barred tiger salamanders (fig. 1). The cysts were randomly distributed, large (2–3 mm diameter), firm and raised ($\leq$1 mm). Each cyst comprised a clear, thin capsule containing an oval-shaped parasite 0.75 × 4.3 mm, with an oral and a ventral sucker (fig. 1). The most common sites for cysts were the inguinal and axillary regions on the anurans and salamanders, followed by the toes and tails of salamanders. Nodules were not noted internally on gross examination, but similar cysts were observed in the liver, heart and neck region of one of the salamanders (fig. 1). In addition to the large cysts, there were multiple randomly distributed small (*c.* 1 mm diameter), subcutaneous, firm, nodules (i.e. cysts) throughout the bodies of all individuals.

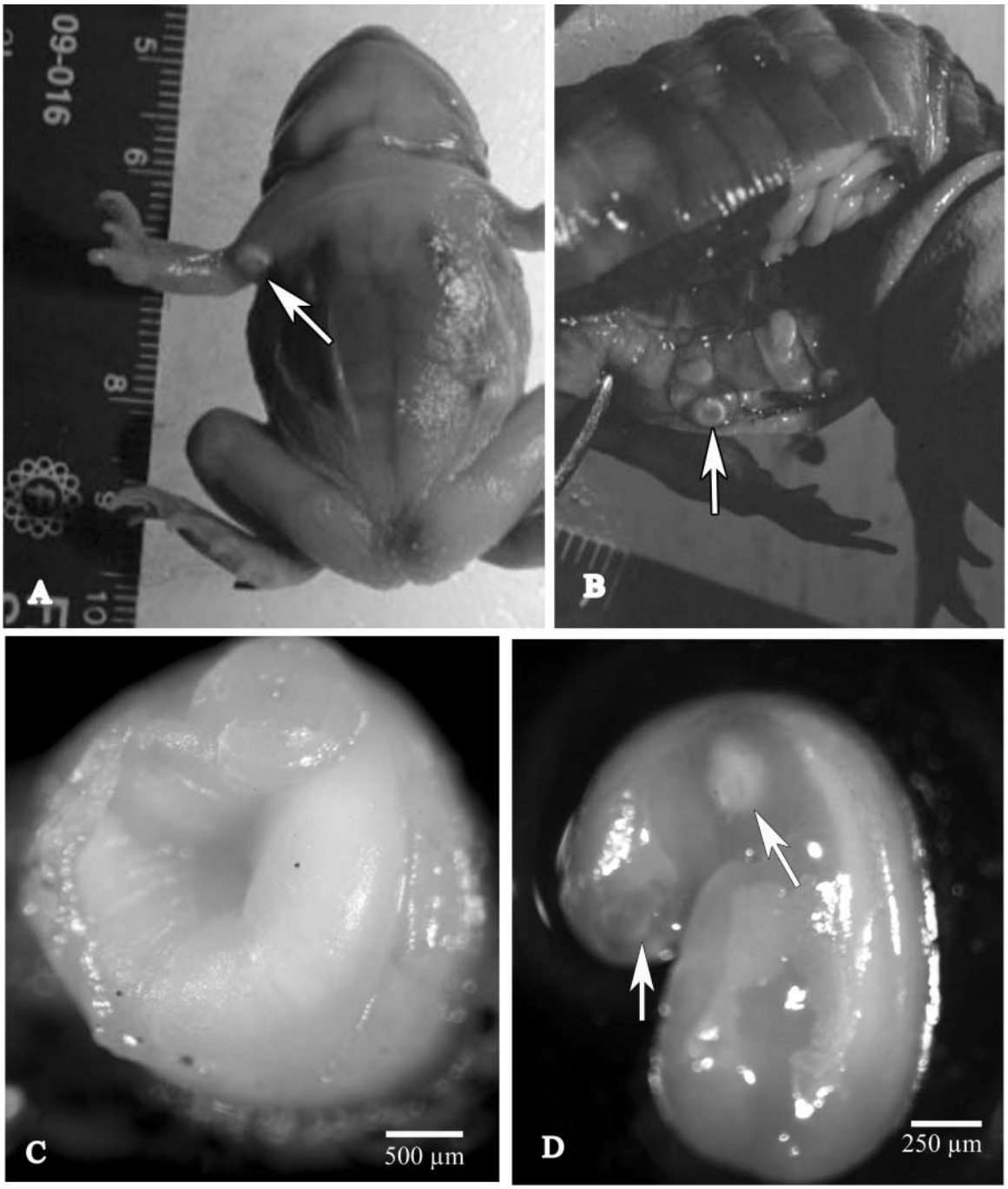

Fig. 1. Subcutaneous firm nodules (arrow) caused by metacercariae of *Clinostomum attenuatum* in a New Mexico spadefoot (*Spea multiplicata*) (A) and a barred tiger salamander (*Ambystoma tigrinum mavortium*) (B) collected from a Texas playa. Cysts (arrows) occurred within subcutaneous tissue (A) but occasionally internal cysts were seen (B). Metacercariae were contained within a clear capsule (C) and had both an oral and a ventral sucker (arrows) (D).

Sections of brain, heart, lung, liver, kidney, pancreas, gastrointestinal tracts, reproductive tissues, spleen, skin and bone from the specimens were paraffin-embedded, sectioned (5 μm) and stained with haematoxylin and eosin for light microscopic examination. Significant pathological changes were rarely associated with the cystic lesions. The anurans contained numerous dermal cysts, each containing a parasite, often extending into the underlying skeletal muscle. Each parasite measured 1.5–2 mm in diameter, with a thin (*c.* 10 μm wide) eosinophilic-staining tegument bearing small nearly indiscernible tegumental spines (fig. 2). In addition,

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posteriorly located primordial genitalia, paired digestive caeca, and an oral and a ventral sucker were present. There were increased numbers of melanomacrophages within the liver, kidneys and occasionally in the spleen. One digenean cyst, surrounded by melanomacrophages, occurred within the renal interstitium of an adult male spadefoot.

Cystic dermal lesions in the salamanders were similar to those in the anurans. One salamander was severely affected with associated tissue changes, including accumulations of melanomacrophages. Numerous digenean cysts were adjacent to, but not infiltrating, the liver and pancreas and within the mesentery surrounding the gastrointestinal tract (fig. 2). An increased number of melanomacrophages was observed within the liver and spleen.

Serial sections were made to identify the organisms contained within the grossly observed small cysts. These cysts were located within the epidermis and rarely within the dermis. Each cyst contained a mite with a dense eosinophilic-staining exterior (i.e. chitin), striated muscle, and jointed appendages. Pathological changes were not observed within the surrounding tissues.

Parasites in the large cysts of the anurans and salamanders were identified as the metacercariae of *Clinostomum attenuatum*. *Clinostomum* spp. are digeneans that, as adults, live in the oral cavity and oesophagus of Ardeidae (McAllister, 1990). Although snails serve as the first intermediate host, fish or amphibians are common second intermediate hosts (McAllister, 1990). Specifically, *C. attenuatum* is considered a digenean of the mouth cavity of bitterns and herons, and frogs are reported as a second intermediate host (Cort, 1913; Ukoli, 1966). Speciation of *Clinostomum* has met with some confusion as previously recognized species from various geographical regions have been discovered to be similar enough to be considered the same species (McAllister, 1990; Matthews & Cribb, 1998). The differential for digeneans in the large cysts of anurans and salamanders would be *Clinostumum complanatum*, often referred to as *C. marginatum* in North America but currently accepted as the same species (McAllister, 1990). In the broadest sense, *C. complanatum* is thought to occur in fish whereas *C. attenuatum* is thought to occur in frogs, although *C. complanatum* has been reported in amphibian species (McAllister, 1990). Similar to Creel *et al*. (2000), we identified the digeneans from our amphibians as *C. attenuatum* rather than *C. complanatum,* based on the placement of the primordial genitalia within the posterior half of the body and the uniformity of the body widths.

Species of *Clinostomum* have been identified from numerous amphibians but reports of parasitized *Bufo cognatus*, *Spea multiplicata* and *Ambystoma tigrinum* have not been published. *Clinostomum* spp. have been primarily reported in Ranidae (Fortner, 1923; Trowbridge & Hefley, 1934; Ingles, 1936; Najarian, 1955; Jinks & Johnson, 1970; Muzzall, 1991b; Goldberg *et al.*, 1998; and Muzzall *et al.*, 2001). Additionally, *C. attenuatum* has been reported in *Bufo marinus* (Etges, 1991) and *Hyla cinerea* (Creel *et al.*, 2000). Few records of *Clinostomum* spp. in salamanders exist and have not included a species of *Ambystoma* (Bennett & Humes, 1938; McAllister, 1990; Muzzall, 1991a). Therefore, this report serves as the first record of *C. attenuatum* in *Ambystoma tigrinum mavortium*, *Bufo cognatus* and *Spea multiplicata*. Voucher specimens have been deposited in the United States National Parasite Collection, Beltsville, Maryland 20705: *Clinostomum attenuatum* Ex: *Bufo cognatus* (94035), *Clinostomum attenuatum* Ex: *Spea multiplicata* (94036), *Clinostomum attenuatum* Ex: *Ambystoma trigrinum mavortium* (94037).

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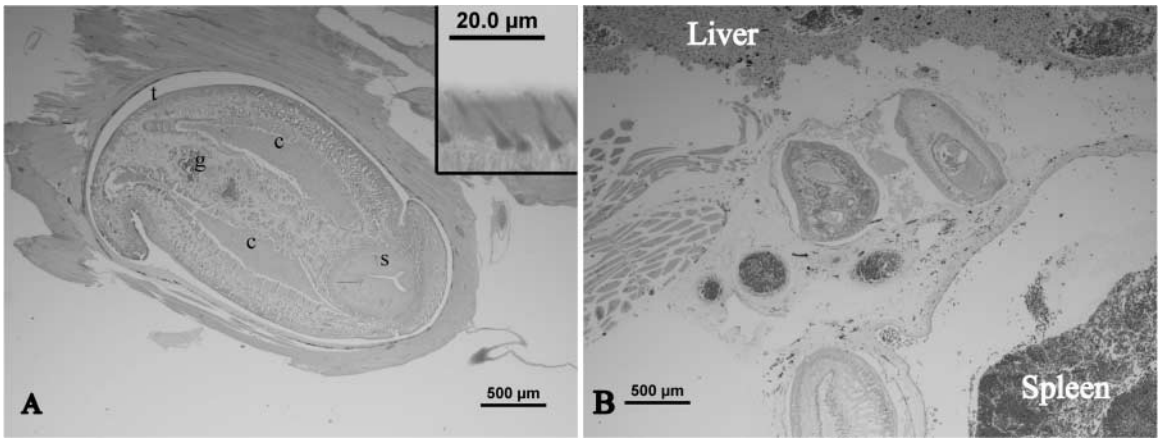

Fig. 2. Metacercariae of *Clinostomum attenuatum* identified in Great Plains toads (*Bufo cognatus*), New Mexico spadefoots (*Spea multiplicata*) and barred tiger salamanders (*Ambystoma tigrinum mavortium*) collected from Texas playas in 1999 and 2000. Metacercariae (A) had thin eosinophilic-staining tegument (t) with nearly indiscernible tegumental spines (inset), posteriorly located primordial genitalia (g), paired digestive caeca (c) and an oral and a ventral sucker (s). One barred salamander had numerous parasitic cysts within the coelomic mesentery (B).

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