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Infection of Spotted Salamanders (*Ambystoma maculatum*) with Ichthyophonus-like Organisms in Virginia

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ABSTRACT: Ichthyophonus-like organisms were found in two free-ranging adult spotted salamanders (*Ambystoma maculatum*) captured within two different vernal ponds in the Virginia Commonwealth University Rice Center for Environmental Life Sciences in Charles City County, Virginia. Histopathologic examination of necropsied specimens revealed large spores, often enclosed by granulomas. These enclosed spores resembled those caused by the fish pathogen *Ichthyophonus hoeferi*. One salamander displayed an externally visible large swelling beneath the jaws. The other lacked macroscopic abnormalities, but histologic sections of ventral muscle revealed early-stage Ichthyophonus-like organisms and minimal granulomatous reactions. This is the first report of Ichthyophonus-like infection of *Ambystoma maculatum* in Virginia.

Key words: *Ambystoma maculatum*, Ichthyophonus-like organisms, salamander disease.

On 5 February 2006, 85 adult Spotted Salamanders (*Ambystoma maculatum*) were captured overnight in vernal pools (N 37.32837°, W 77.19576°) located at Virginia Commonwealth University's Rice Center for Environmental Life Sciences in Charles City County, Virginia, as part of an initial survey of the amphibian population in these vernal pools. The salamanders were measured, photographed, and released as part of an ongoing population survey. One of the specimens was moribund and displayed a swelling beneath the throat and jaws. Following euthanasia, the skin was opened, and gross examination revealed the swelling measured 11 mm by 5 mm. Histologic examination of this tissue swelling revealed numerous and often confluent granulomas. Approximately 50% of the swelling was due to granulomitous inflammation, with the Ichthyophonus-like organisms at the centers. Many of these granulomas surrounded intact or fragmented spores (Fig. 1) that appeared similar to *Ichthyophonus hoferi*, a pathogen responsible for significant mortality among both freshwater and marine fish (Kocan et al., 1999). Ichthyophonus-like organisms are members of the Class Mesomycetozoa, at the interface of fungi and protozoa (Mendoza et al., 2002). The spores ranged from 140 to 240 μm in diameter. In addition, some granulomas contained fragments of the spore walls phagocytized by foreign body giant cells. The entire area of each spore stained strongly positive with Periodic acid-Schiff reaction. Spore walls also were stained. The wall of each spore stained intensely positive with Gomori methenamine silver stain, as did portions of the interior of the spore. The liver was completely black on gross examination; based on histopatholgy, this was probably due to increased numbers of melanomacrophages interspersed with the hepatocytes. Eyes, lungs, heart, dorsal and ventral skin, ventral muscle, tail muscle, and liver were grossly and microscopically normal.

Gross lesions were not observed in three other adult salamanders that died in traps, and these probably died from suffocation, due to the large numbers of salamanders within the trap. However, one of these three also displayed an early-stage Ichthyophonus-like infection in the muscles lining its ventral side (Fig. 2); no

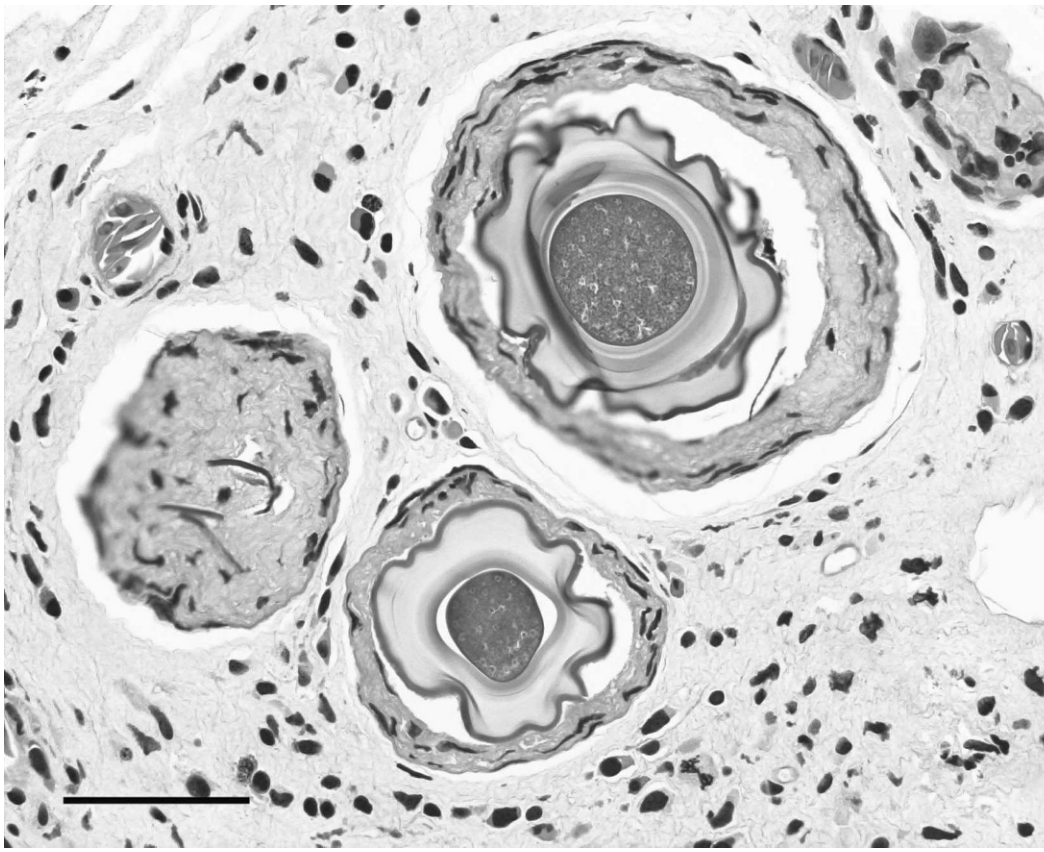

FIGURE 1. Three Ichthyophonus-like spores surrounded by granulomatous inflammation. Throat and lower jaws of this *Ambystoma maculatum* (specimen no. 1, 28 g, 11 mm SVL, 21 mm total length) were significantly swollen due to the granulomatous reaction. The leftmost of the three Ichthyophonus-like spores was partially collapsed and disintegrated. Measurement bar = 100 μm (H&E, 200×).

organisms were observed in eyes, lungs, heart, dorsal and ventral skin, tail muscle, leg muscle, and liver of this animal. The granulomatous reaction was minimal at the time of the death, and this early-stage Ichthyophonus-like infection was not the cause of death. No Ichthyophonus-like organisms were observed in histologic sections of the ventral muscle, tail muscle, leg muscle, liver, heart, or lungs of the two other salamanders. Thus two of four salamanders examined exhibited evidence of the Ichthyophonus-like spores in muscle.

Ichthyophonus-like organisms have been reported in red-spotted newts (*Notophthalmus viridescens*) in West Virginia (Herman, 1984) and in Vermont (Green et al., 1995). Furthermore, Ichthyophonosis appears to be enzootic in Quebec, Canada, where a variety of frogs and one red-spotted newt displayed infection with this organism (Mikaelian et al., 2000). To our knowledge, this is the first report of the infection of adult *Ambystoma maculata* by Ichthyophonus-like organisms in Virginia. As noted by other investigators (Green et al., 2002), information on the presence of these organisms in clinically normal amphibians in the USA is not available. Thus it is not known whether these Ichthyophonus-like organisms are endemic or new to Virginia and surrounding areas, or whether they could exert a significant effect on amphibian populations.

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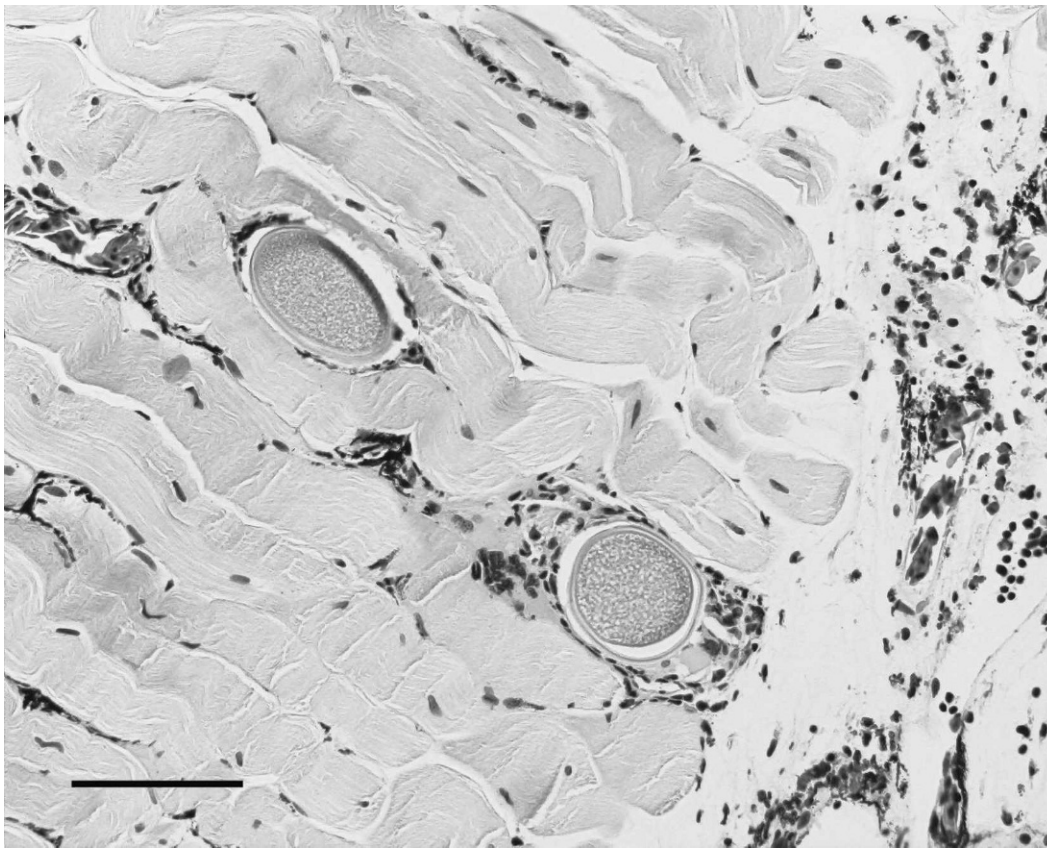

FIGURE 2. Ventral muscles of *Ambystoma maculatum* specimen number 85 (25 g, 10.5 mm SVL, 20.5 mm total length) contained Ichthyophonus-like organisms less mature than those illustrated in Figure 1. The granulomatous reaction was minimal. No macroscopic abnormalities observed. Measurement bar = 200 μm (H&E, 100×).

the Histopathology Laboratory, Department of Pathology, VCU School of Medicine, for preparation of the histologic specimens.

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