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Evidence of Vertical Transmission of the Snake Fungal Pathogen *Ophidiomyces ophiodiicola*

Anne G. Stengle,[1] Terence M. Farrell,[2] Keenan S. Freitas,[3] Craig M. Lind,[4] Steven J. Price,[5] Brian O. Butler,[6] Tigran Tadevosyan,[6] Marcos Isidoro-Ayza,[7,8] Daniel R. Taylor,[7] Megan Winzeler,[7] and Jeffrey M. Lorch[7,9] [1]Science, Engineering and Mathematics Department, Holyoke Community College, 303 Homestead Ave., Holyoke, Massachusetts 01040, USA; [2]Department of Biology, Stetson University, 421 N Woodland Blvd., DeLand, Florida 32723, USA; [3]Lees-McRae College, 191 Main St., Banner Elk, North Carolina 28604, USA; [4]Department of Natural Sciences and Mathematics, Stockton University, 101 Vera King Farris Dr., Galloway, New Jersey 08201, USA; [5]Department of Forestry and Natural Resources, University of Kentucky, T. P. Cooper Building, Room 214, Lexington, Kentucky 40546, USA; [6]Oxbow Associates, Inc., 629 Massachusetts Ave., Suite 201, Boxborough, Massachusetts 01719, USA; [7]US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA; [8]Department of Pathobiological Sciences, University of Wisconsin–Madison, 2015 Linden Drive, Madison, Wisconsin 53706, USA; [9]Corresponding author (email: jlorch@usgs.gov)

ABSTRACT: Snake fungal disease (ophidiomycosis) is an emerging infection of snakes caused by *Ophidiomyces ophiodiicola*. Little is known about mechanisms of this pathogen's transmission and its implications for conservation of wild snake populations. We report four cases with evidence of vertical transmission of *O. ophiodiicola* from dam to offspring.

Understanding pathogen transmission routes is critical in elucidating epidemiology and managing disease in wildlife populations. Snake fungal disease (SFD, ophidiomycosis), caused by *Ophidiomyces ophiodiicola* (Oo), is an emerging threat to some snake populations in the eastern US (Allender et al. 2011; Lorch et al. 2015, 2016). Transmission routes for Oo remain poorly known, a gap in our understanding of SFD ecology (Allender et al. 2015). Here we present four cases that may represent examples of vertical transmission as defined by Mims (1981) to include postnatal transmission of Oo.

A gravid queensnake (*Regina septemvittata*) with skin lesions was captured in Fayette County, Kentucky, US, on 27 July 2015. *Ophidiomyces ophiodiicola* was detected on a swab of lesioned skin by real-time PCR (Bohuski et al. 2015). Skin lesions remained when the snake was recaptured 14 d later and brought into the laboratory. Three days later, she gave birth to 13 offspring. The skin of each neonate was swabbed within 12 h after birth, and all were PCR-positive for Oo. The offspring exhibited no signs of disease, and the dam and her offspring were released at the capture site.

A gravid eastern milksnake (*Lampropeltis triangulum triangulum*) exhibiting gross lesions consistent with SFD (Lorch et al. 2015) was captured and taken into captivity on 18 June 2016 in Norfolk County, Massachusetts, US. *Ophidiomyces ophiodiicola* was cultured from lesions on molted skin 40 d after capture and identified through DNA sequencing (Lorch et al. 2016). On 5 July 2016, the snake laid 18 eggs, which were transferred to an incubator within 6 h and maintained at 24–26 C. Within 10 d of incubation, shells of five eggs exhibited thick cottony white fungal growth. By day 21, all eggs had become moldy and none hatched. After 66 d of incubation, the eggs were swabbed. Eight eggs were PCR-positive for Oo. Attempts to culture Oo (Lorch et al. 2016) were unsuccessful, likely due to the abundance of saprophytic fungi on the eggs.

On 2 July 2016, a gravid eastern kingsnake (*Lampropeltis getula*) with skin lesions consistent with SFD (Fig. 1A) was captured in Halifax County, North Carolina, US, and brought into captivity for rehabilitation (i.e., supportive care). After 5 d, the snake molted and laid 10 eggs; the eggs were immediately transferred to a separate container for incubation. The skin lesions appeared to resolve with molting, and the dam was released at the site of capture. Six young hatched after 57–58 d (one egg produced twins); the remaining five eggs failed to hatch. Within 4–5 d of

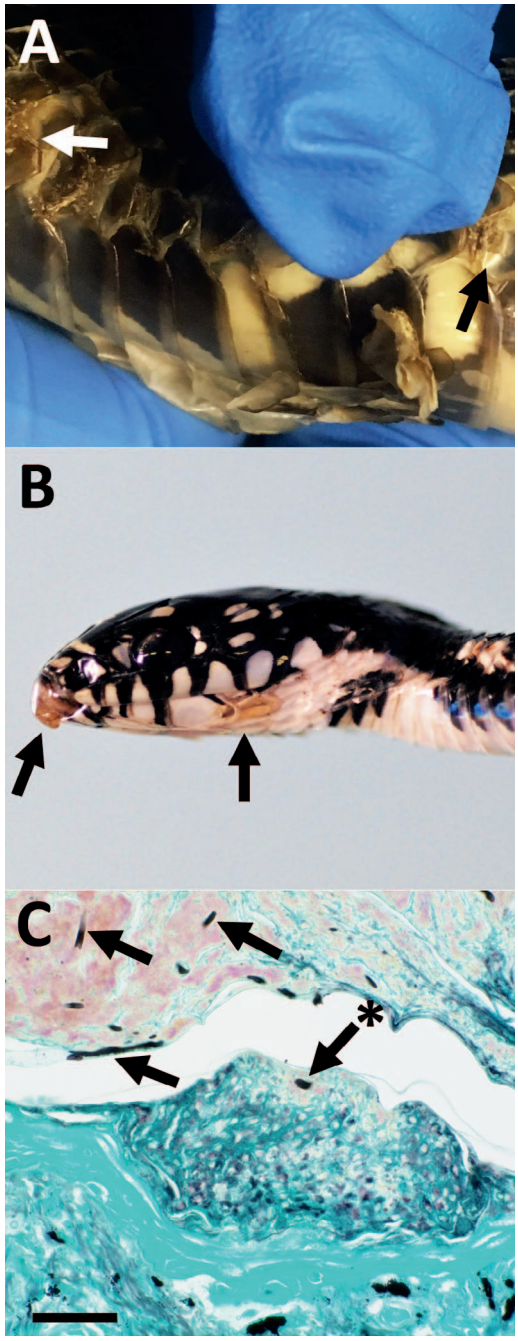

FIGURE 1. *Ophidiomyces ophiodiicola* infection of an adult female eastern kingsnake (*Lampropeltis getula*) collected on 2 July 2016 in North Carolina, USA, and her offspring. (A) The dam exhibited clinical signs of snake fungal disease (SFD; arrows) at the time of capture. (B) Clinical signs of SFD (arrows) developed in the offspring within 4–5 d of hatching. (C) Histopathologic lesions of SFD in the neonatal kingsnake (Grocott's methenamine silver stain). ← Arrows point to examples of numerous fungal hyphae in the portion of the skin about to be molted. A hypha can also be seen within a necrotic region of the new epidermis (arrow with asterisk). Scale bar=40 μm.

hatching, the neonates developed skin lesions (Fig. 1B). Despite molting, lesions recurred, and all six snakes died 15–80 d after hatching. Histopathologic lesions suggestive of SFD (Lorch et al. 2015) were observed in skin of all five neonates (Fig. 1C) that were examined, and Oo was grown in culture (n=4) or detected by PCR (n=5). However, an ultimate cause of death was not determined. Swabs from nine of the 10 egg shells were PCR-positive for the fungus, and viable Oo was recovered from one of the eggs (cultures of the others were overwhelmed by saprophytic fungi).

On 6 June 2017, a gravid pygmy rattlesnake (*Sistrurus miliarius*) was captured in Volusia County, Florida, US, and held in a field enclosure for a separate study. The snake did not exhibit clinical signs of SFD upon capture but had developed skin lesions by 14 August 2017 when she gave birth to four offspring. The lesions on the female were not swabbed for testing. Skin lesions manifested in two of the young 9 d after birth. One of these two neonates tested positive for Oo by real-time PCR; the second tested negative for the fungus. All snakes were released at the point of capture prior to knowing the PCR results.

Vertical transmission of fungal pathogens has occasionally been reported in humans and is primarily thought to occur during or after birth (Battin and Wilson 2005; Bliss et al. 2008). In the case of Oo, it seems most plausible that young snakes of viviparous species (e.g., queensnake, pygmy rattlesnake) are exposed to the fungus by contacting areas of infected skin of the dam after birth. In oviparous snake species (e.g., eastern kingsnake, eastern milksnake), hatchlings may be exposed via eggshells or nesting cavities contaminated with Oo conidia left behind by an infected dam.

Examples of transovarian or transplacental transmission of fungal pathogens are rare

(Cere et al. 1997; Whitt et al. 2004), and we are unaware of examples involving dermatophytes or fungi closely related to *Ophidiomyces*. We screened swabs of the reproductive tract of a dead gravid female pygmy rattlesnake with SFD collected in Volusia County, Florida, as well as the chorion, yolk sac, and skin surface of three late-term embryos she was carrying, for the presence of Oo using PCR. All samples were negative. However, Oo was detected on a swab of the cloacal mucosa of the female, suggesting that the young could have become exposed during birth. We did not uncover evidence of potential transovarian transmission as has been reported with a *Hepatozoon* parasite and reptarenaviruses in snakes (Kauffman et al. 2017; Keller et al. 2017). However, additional sampling is needed to rule out transovarian transmission.

The four cases presented here provided circumstantial evidence that Oo may be passed from female snakes to offspring. Although we used clean cages and substrates (e.g., newspaper, aspen shavings, vermiculite) for husbandry and incubation that were unlikely to be contaminated, substrates were not screened for the presence of Oo, and thus we cannot definitively rule out environmental transmission. Furthermore, because our cases were incidental and isolated, we cannot confirm the role of vertical transmission in SFD without a more rigorous laboratory study. However, the potential for Oo to be spread in this manner highlights the importance of pathogen screening, implementing biosecurity measures for captive breeding programs aimed at recovery of rare snake species, and the need to track the health and fate of snakes born from females harboring Oo.

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