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Snake Fungal Disease in Colubridae Snakes in Connecticut, USA in 2015 and 2017

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ABSTRACT: Snake fungal disease (SFD), caused by the fungus *Ophidiomyces ophiodiicola*, is an emerging threat to wild snake populations in the US. Data regarding its distribution, prevalence, and population-level impacts are sparse, and more information is needed to better manage SFD in the wild. In this study, we captured 38 wild snakes of five species in Connecticut in the summers of 2015 and 2017. Skin lesions were biopsied and evaluated histologically for fungal dermatitis. At least one individual from each species was positive for SFD, and 48% of snakes sampled in 2015 and 39% of snakes sampled in 2017 were positive for SFD. A Dekay's brownsnake (*Storeria dekayi dekayi*) with SFD lesions, captured in the summer of 2017, extended the host range of the disease. Thus, SFD was present in wild Connecticut snakes in 2015 and 2017, which demonstrated a wide-spread distribution throughout the state.

Key words: Colubridae, emerging infectious disease, *Ophidiomyces ophiodiicola*, snake fungal disease.

Snake fungal disease (SFD) has been documented in over 30 snake species across most of eastern North America since 2006 (Lorch et al. 2016). Yet there is limited data to provide insight into the emergence of this infectious disease and its long-term impacts on snake populations. *Ophidiomyces ophiodiicola* is the fungus responsible for SFD, which is characterized by skin lesions and, in more-severe cases, lesions in lung, muscle, and bone (Lorch et al. 2015; Allender et al. 2016). In a laboratory study, infected snakes shed their skin more often, a defense against infection that is energetically costly. Behavioral changes observed in snakes affected with SFD include bouts of anorexia which may decrease overall body condition. Infected snakes in captivity exhibit extended periods of time in open or conspicuous areas, although infected snakes in the wild show the opposite behavior, spending less time exposed than do noninfected snakes (Lorch et al. 2015; Tetzlaff et al. 2017). The behavioral impacts of SFD are not fully understood, so further investigations are important.

The emergence of SFD has led to declines in wild snake populations, perhaps most notably in the 2006 outbreak in New Hampshire where more than half the population of timber rattlesnakes (*Crotalus horridus*) was lost (Clark et al. 2011). The SFD has been reported in *C. horridus* in Massachusetts as well, and work is underway to confirm the presence of SFD in other *C. horridus* populations across New England (McBride et al. 2015). Additionally, SFD has recently been confirmed in wild European snake populations (Franklinos et al. 2017), in six different families, including the large family Colubridae (Lorch et al. 2016). With such a large geographic range and extensive host compatibility, studying this emerging threat is an important topic for snake ecology.

Snakes were opportunistically captured using visual encounter surveys at sites in towns throughout Connecticut (Hamden, Berlin, Haddam, Burlington, Bethel, Killingworth, Kent, Middletown, Meriden, Middlefield, and East Haddam). Field technicians recorded mass to the nearest gram, snout-to-vent and vent-to-tail measurements to the nearest centimeter, age class determination (juvenile, subadult, and adult), and sex. If SFD was suspected on a captured snake based

TABLE 1. Species of snakes captured in Connecticut in the summers of 2015 and 2017 and tested for snake fungal disease (SFD). Criteria for classifying snakes as positive for SFD were the presence of fungal dermatitis under histologic tissue analysis (H&E and Grocott's methenamine blue stains) or by positive PCR result. The SFD-negative snakes showed no fungal dermatitis and were PCR negative. NA = not applicable, as these species were not targeted in the 2015 season.

Snake	Species	2015		2017	
		No. captured	No. positive	No. captured	No. positive
Northern watersnake	*Nerodia sipedon sipedon*	6	4	13	5
Northern black racer	*Coluber constrictor constrictor*	7	2	2	1
Dekay's brownsnake	*Storeria dekayi dekayi*	NA	NA	1	1
Eastern gartersnake	*Thamnophis sirtalis sirtalis*	NA	NA	3	1
Eastern ratsnake	*Pantherophis alleghaniensis*	NA	NA	6	1

on presence of lesions, the locations of lesions were recorded and photographs were taken. All field studies and samples were taken with Institutional Animal Care and Use Committee approval from Mystic Aquarium, Mystic, Connecticut (no. 14007).

The protocol for collection of skin biopsies followed the protocol developed for the Snake Fungal Disease Regional Conservation Needs State Wildlife Grant Program (Bauder et al. 2016). Biopsies of skin lesions were obtained using sterile surgical techniques. Local anesthesia was established by infiltrating the area with lidocaine (Lidocaine-2%, Phoenix, St. Joseph, Missouri, USA) diluted to 0.5%. A 3-mm punch biopsy tool (Miltex, York, Pennsylvania, USA) was used to obtain the biopsy specimen, which was then removed from the site using sterile forceps and scissors. Each biopsy site was closed using 3-0 polydioxanone suture (Ethicon, Johnson & Johnson, Cincinnati, Ohio, USA) in a cruciate pattern. One half of the biopsy specimen was placed into 10% buffered formalin for histopathology while the second half of the biopsy specimen was placed in a sterile plastic bag and stored in a 4 C refrigerator until shipment. All snakes with or without suspect fungal lesions were swabbed from head to tail along the dorsal, lateral, and ventral surfaces of the body using a polyester swab. The swab was then placed into a sterile plastic bag and stored at room temperature until shipment. Samples were shipped overnight to the Connecticut Veterinary Medical Diagnostic Laboratory (Storrs, Connecticut) for histologic processing and evaluation by a veterinary pathologist. Samples were considered SFD-positive if fungal dermatitis was observed histologically (H&E and Grocott's methenamine blue stains) in tissue samples from a snake lesion or from a positive PCR result (as described by White et al. 1990 and Romanelli et al. 2010). Snakes with no observable lesions and a negative PCR were considered SFD-negative. Positive PCR results were secondary criteria for positive snakes where fungal dermatitis was observed histologically due to the significant chance of false negatives in conventional PCR (Allender et al. 2015; Hileman et al. 2018). Fungal culture (potato dextrose agar), intergenic sequence PCR, and sequencing on suspect-positive isolates for genus and species ID were conducted at the University of Florida Veterinary Diagnostic Laboratories (Gainesville, Florida).

A total of 13 (two species) and 25 (five species) native colubrid snakes were sampled and tested for SFD in the summers of 2015 and 2017 (Table 1). The snake species sampled were the northern black racer (*Coluber constrictor constrictor*), northern watersnake (*Nerodia sipedon sipedon*), eastern ratsnake (*Pantherophis alleghaniensis*), eastern gartersnake (*Thamnophis sirtalis sirtalis*), and Dekay's brownsnake (*Storeria dekayi dekayi*). Samples tested from northern watersnakes were positive in five of 13 snakes

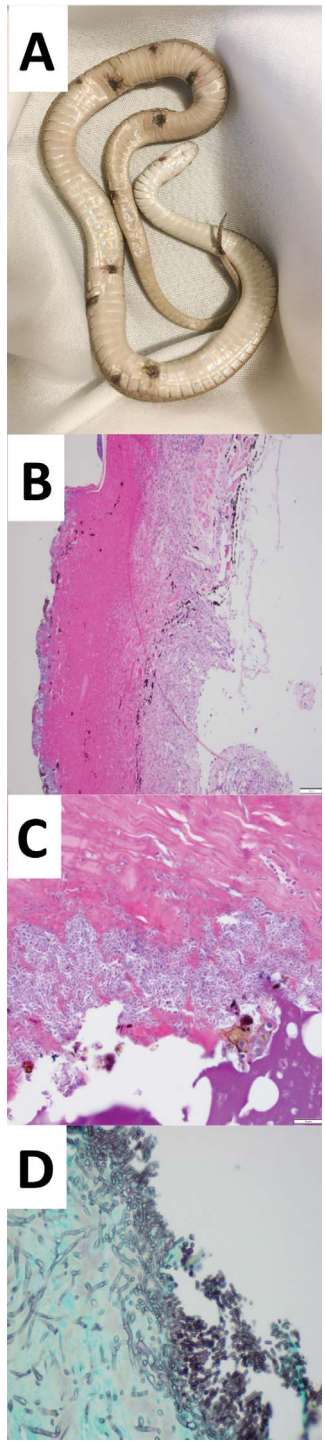

FIGURE 1. Snake fungal disease lesions in a northern brownsnake (*Storeria dekayi dekayi*) captured in Connecticut, USA in the summer of 2017. (A) Gross lesions consisted of multiple and discrete foci of darkening and crusting in the skin of the ventrum. (B) Microscopically, lesions exhibited marked eosinophilic crusting and necrosis of the epidermis and superficial dermis. H&E stain. Bar=50 µm. (C) Higher magnification shows fungal hyphae within the crust and more-prominently along the surface. H&E stain. Bar=20 µm. (D) Fungal hyphae and arthroconidia accentuated with Grocott's methenamine silver stain. Bar=20 µm.

in 2017 and four of six in 2015. One of two northern black racers tested positive in 2017 and two of seven were positive in 2015. During the 2017 season we sampled snake species in addition to northern black racers and northern watersnakes. We detected SFD in one of six eastern ratsnakes, one of three eastern gartersnakes, and a single Dekay's brownsnake. The majority of lesions on SFD-positive snakes were found on the ventral surface of the body, with an absence of lesions on the head or face, which has been common in vipridae species (Clark et al. 2011; McBride et al. 2015; Allender et al. 2016). The Dekay's brownsnake was severely affected with multiple lesions across both the ventral (Fig. 1A) and dorsal body surface and died shortly after giving birth to three live and 10 stillborn neonates. The neonates were not tested for SFD and no lesions were found upon external examination; they were released back into the wild. The dead female snake was necropsied, and lesion and tissue samples were taken and examined histologically. Prominent eosinophilic crusts covered infected areas (Fig. 1B), with fungal hyphae sometimes extending into underlying muscle (Fig. 1C). In one severe lesion, the fungal hyphae infiltrated so deep as to cause a coelomitis. Fungal plating of a swab taken from a lesion of the Dekay's brownsnake (Fig. 2A) resulted in the growth of a powdery, yellow-white colony that was typical of *O. ophiodiicola* (Okhura et al. 2016). Fungal hyphae were apparent when fungal cells were observed by light microscopy (Fig. 2B). An amplicon from an SFD-specific PCR from the fungal colony was sequenced and identified as *O. ophiodiicola*. Our results extended the host range of SFD to Dekay's brownsnakes.

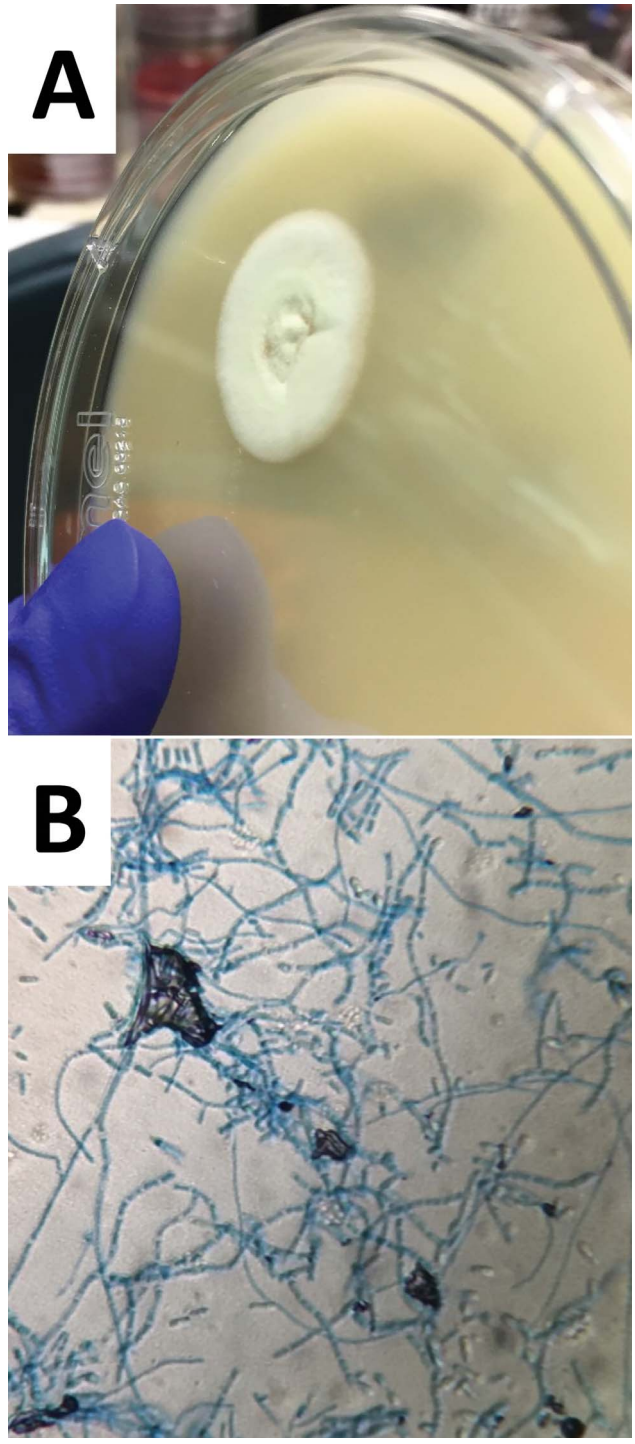

FIGURE 2. A fungal isolate from a case of snake fungal disease derived from a snake captured in Connecticut, USA. (A) Fungal colony identified by PCR and sequencing as *Ophidiomyces ophiodiicola*, growing on potato dextrose agar. (B) Fungal hyphae from the culture observed under a light microscope (40×).

The extent of disease in the severely diseased Dekay's brownsnake snake, and the potential that its lesions could have been exacerbated by its concurrent pregnancy (due to additional metabolic strain) and potentially contributed to its death, raises questions about what host factors may lead to susceptibility to SFD infection and mortality. There appears to be no relationship between the severity of SFD and reproductive status in female pigmy rattlesnakes (*Sistrurus miliarius*) during breeding season; however, these snakes have a lower average severity of disease in other seasons if they are pregnant or vitellogenic (McCoy et al. 2017). Further studies in this area are important to consider a possible relationship of host factors and SFD severity or susceptibility in viviparous and oviparous species (Burbrink et al. 2017).

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