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Quantification of Infection Prevalence in a Multi-Species Snake Community Following Detection of the First Confirmed Case of Ophidiomycosis in Rhode Island, USA

Ophidiomycosis is an infectious disease caused by the fungal pathogen *Ophidiomyces ophidiicola*. Infections resulting from this pathogen have been observed in 49 native and three non-native snake species in North America (Haynes and Allender 2021). Following external colonization of its host, *O. ophidiicola* penetrates the keratinized outer skin layer, spreading into the epidermis through open lesions and causing discoloration and eventually the formation of a brown crust on affected scales (Lorch et al. 2015; Haynes and Allender 2021). Once internalized, the fungus may further invade and disfigure organs, muscle tissue, and/or bone (Allender et al. 2011; Haynes and Allender 2021). Evidence from case reports and experimental infection studies suggests that *O. ophidiicola* can be transmitted both vertically (Stengle et al. 2019) and horizontally (McKenzie et al. 2020a) among conspecifics, as well as through contaminated soil in hibernacula and surface environments (Campbell et al. 2021). While the average ophidiomycosis-associated mortality rate in infected wild populations has been estimated at approximately 10% (Davy et al. 2021), mortality rates of 50% or more have been documented within groups of infected individuals both in the wild (Allender et al. 2011; Clark et al. 2011) and in captivity (McKenzie et al. 2020a). These high-mortality cases are likely the exception rather than the rule and may result from interaction of the disease with additional stressors such as inbreeding, climate change, or inadequate captive husbandry. Currently, wild snakes infected with *O. ophidiicola* have been reported from 26 US states, Puerto Rico, and Ontario, Canada (Haynes and Allender 2021). Herein, we provide two new *O. ophidiicola* case reports and results of subsequent field surveillance to assess *O. ophidiicola* prevalence in Rhode Island, USA.

At 1300 h on 17 May 2021, an adult female Northern Watersnake (*Nerodia sipedon*) with a thick brown crust over both eye caps (Fig. 1A) was captured near an artificial pond in Kingston, Rhode Island, USA (41.4973°N, 71.5312°W; WGS 84). A qPCR test run on a swab sample collected from the snake returned positive for *O. ophidiicola* and the snake was retained for treatment at the Wildlife Clinic of Rhode Island. To our knowledge, this observation constitutes the first published report of ophidiomycosis in the state of Rhode Island (Haynes and Allender 2021). Over the course of the next four months, the infected snake was treated with nebulized terbinafine (an antifungal) and neomycin-bacitracin-poly-HC ointment (to prevent secondary bacterial infections). Following treatment, the snake displayed clear eye caps (Fig. 1B), tested negative for *O. ophidiicola* (as assessed via swab-based qPCR), and was released at the original site of capture at 1045 h on 15 September 2021.

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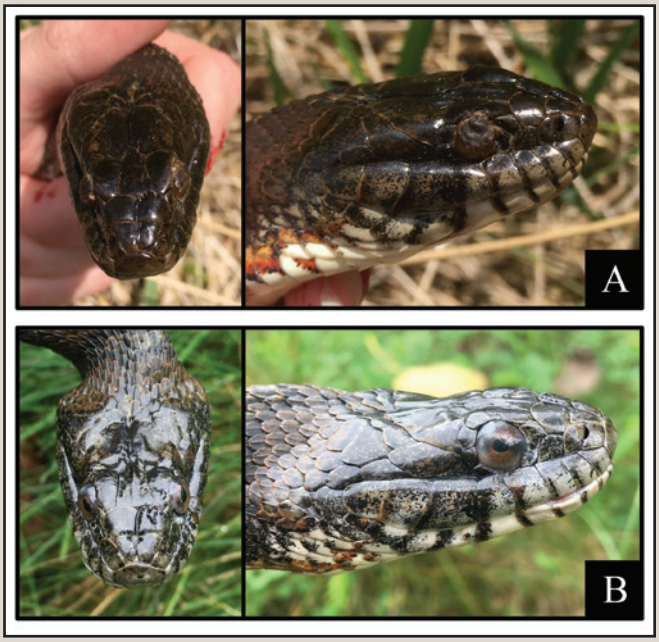

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FIG. 1. *Ophidiomyces*-infected Northern Watersnake (*Nerodia sipedon*) from Rhode Island, USA before (A) and after (B) treatment with terbinafine.

TABLE 1. *Ophidiomyces ophidiicola* occurrence (assessed via qPCR) among three snake species from Rhode Island, USA.

Species	N	# *O. ophidiicola* positive	# *O. ophidiicola* negative	% positive	Total DNA (ng) – positive samples only minimum	mean	maximum	standard deviation
Nerodia sipedon	8	7	1	87.5	0.51	548	1,807	729
Thamnophis sauritus	11	3	8	27.3	0.05	0.41	0.93	0.46
Thamnophis sirtalis	11	5	6	45.5	24	6,960	21,487	8,684
All species pooled	30	15	15	50.0	0.05	2,576	21,487	5,667

A second female watersnake captured on 7 July 2021 in Harrisville, Rhode Island (41.9686°N, 71.6396°W; WGS 84) also displayed visible lesions and tested positive for *O. ophidiicola*, indicating that the presence of this pathogen in Rhode Island is not confined to a single locale. This individual was also successfully treated with terbinafine, and a negative qPCR test result was obtained from a swab sample collected at the conclusion of treatment. The snake was released on 26 September 2021 along with 16 offspring born during the treatment period.

The site from which the first infected individual was captured is inhabited by several snake species, most of which occur there at relatively high densities. In response to our discovery, we initiated a series of surveys to quantify the prevalence of *O. ophidiicola* within this multi-species community. Northern Watersnakes (*Nerodia sipedon*), Eastern Ribbonsnakes (*Thamnophis sauritus*), and Eastern Gartersnakes (*Thamnophis sirtalis*) were located and captured by hand over the course of 22 visual encounter surveys conducted between 13 August 2021 and 15 October 2021 in Kingston, Rhode Island (41.4979°N, 71.5311°W; WGS 84). The only additional snake species encountered but not sampled due to low abundance was Dekay's Brownsnake (*Storeria dekayi*); only two *S. dekayi* were encountered during the study, neither of which displayed external lesions. Each snake captured was inspected for visible lesions, swabbed for *O. ophidiicola*, weighed, measured (snout-vent length), sexed (via probe), photographed, and marked with visible implant elastomer to avoid repeat sampling. Swab samples were collected by passing a swab three times each over the left, right, and ventral surfaces of each snake, then over the mouth, cloacal scale, and vigorously over any visible lesions. To minimize the likelihood of disease transmission among individuals, clean nitrile gloves were worn at all times while collecting and processing snakes and were changed after each release. Non-disposable equipment was decontaminated using either a 10% bleach solution or wipes saturated in 70% isopropyl alcohol (as appropriate) after processing each individual (Rzadkowska et al. 2016). Footwear was thoroughly bleached and rinsed at the conclusion of each survey. All snakes sampled (including those with visible lesions) were released at the site of capture immediately following data collection.

Swabs were collected from snakes of all life history stages, but the subset of samples selected for testing (30 of 40 total) included only the largest individuals (determined by SVL) within each species group. This selection criterion was applied based on the assumption that large adult snakes would be more likely to carry or be infected with *O. ophidiicola* than small juveniles, as none of the smallest snakes in our sampling group displayed visible external lesions. Frozen swabs were shipped to the veterinary diagnostic laboratory of the University of Illinois College of Veterinary Medicine and tested for *O. ophidiicola* via qPCR (see Allender et. al. 2015 for *O. ophidiicola*-specific qPCR protocol).

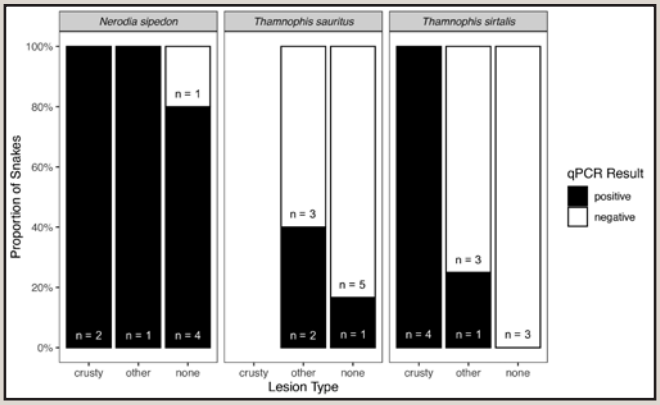

FIG. 2. Visible lesion status versus *Ophidiomyces ophidiicola* test result for *Nerodia sipedon*, *Thamnophis sauritus*, and *Thamnophis sirtalis* from Rhode Island, USA. Lesions categorized as "crusty" are suggestive of ophidiomycosis and consistent with the "necrotic scale" designation of Baker et al. (2019). The "other" category encompasses a variety of alternate lesion types, including scarring, injury, and/or minor scale discoloration.

Of the 30 snakes assessed for ophidiomycosis during our surveys, 15 (50%) tested positive for *O. ophidiicola* (Table 1) and 6 of these (20%) also displayed crusty brown lesions suggestive of ophidiomycosis. Proportions of infected individuals differed by species (*N. sipedon*: 7 [87.5%]; *T. sauritus*: 3 [27.3%]; *T. sirtalis*: 5 [45.5%]). All snakes with skin lesions characterized by brown crusting tested positive, while test results for snakes without visible lesions and those with alternate lesion types (scarring, mild discoloration, etc.) were mixed (Fig. 2). Among individuals that tested positive, qPCR tests returned the highest quantities of DNA (reported per ng of total DNA) for samples collected from snakes showing the most advanced external symptoms of ophidiomycosis. These individuals displayed crusty lesions consistent with the "necrotic scale" category as defined by Baker et al. (2019), although no snake exceeded a clinical sign score of 1 (= low symptom level) based on the criteria of McCoy et al. (2017). We strongly suspect the absence of individuals displaying more extreme symptoms (snakes with upwards of five lesions, facial and cloacal lesions, etc.) was due to our choice to conduct surveys in the fall. Symptoms of ophidiomycosis are often most severe when snakes emerge from brumation in the spring (Haynes and Allender 2021; McKenzie et al. 2020b), so our results should be considered a conservative reflection of population disease status during what is likely the "healthiest" time of year. We provide examples of lesions observed on snakes that tested positive for *O. ophidiicola* as well as from snakes that tested negative (Fig. 3); because swabs are capable of collecting fungal DNA only when it is present externally, it is possible that lesions associated with negative test results may still be the result of an internal fungal infection.

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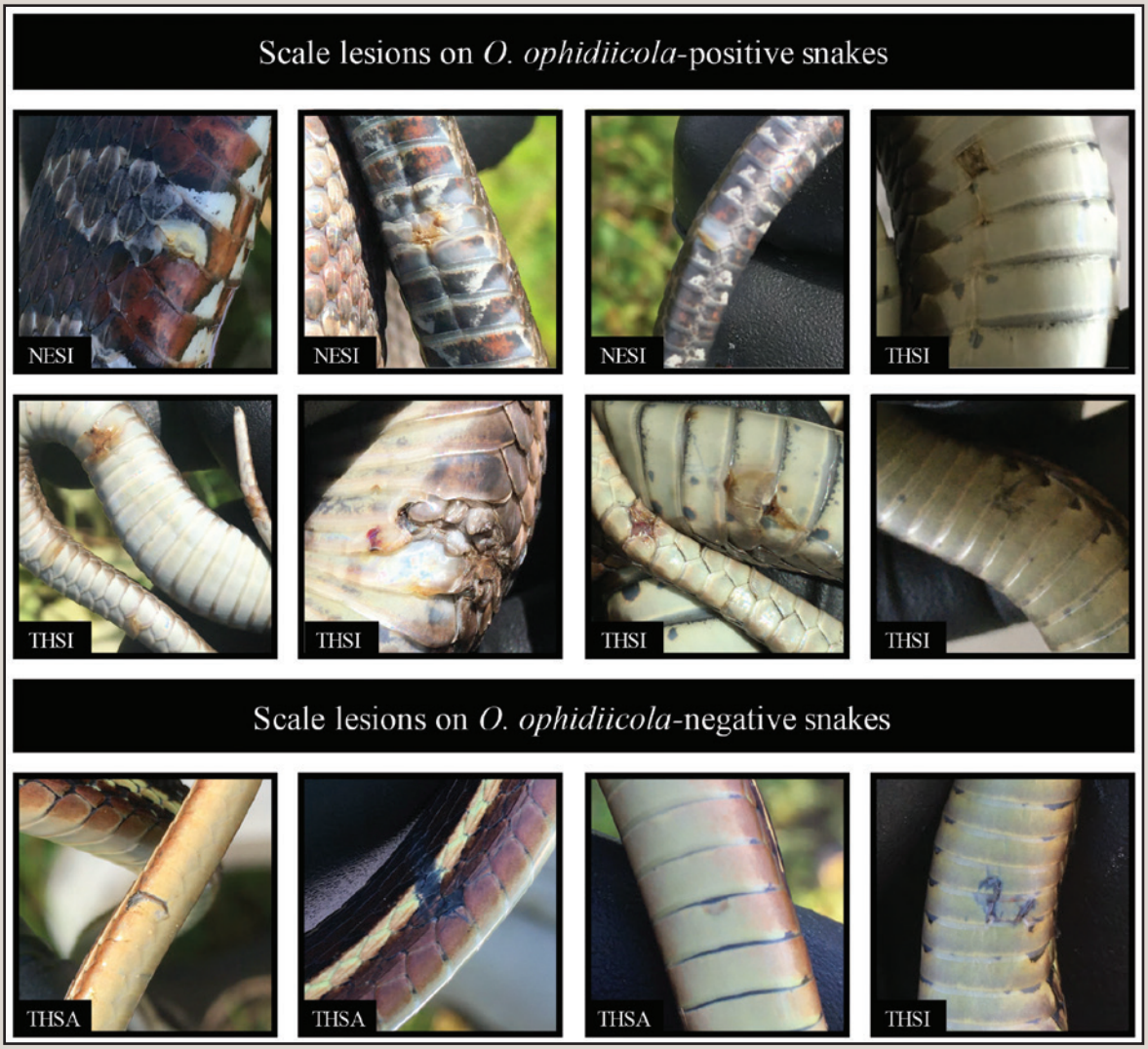

FIG. 3. Contrasting examples of lesions occurring on snakes that tested either positive or negative for *Ophidiomyces ophidiicola* (as assessed via swab-based qPCR) in Rhode Island, USA. Species is indicated in the lower left corner of each image with a four-letter code (*Nerodia sipedon* = NESI, *Thamnophis sauritus* = THSA, *Thamnophis sirtalis* = THSI).

An exploratory investigation of the proportional relationships between qPCR test results and both sex and body condition (calculated as the residual of a mass–SVL regression) revealed no obvious patterns of association among these variables. Intraspecies sample sizes were too low to permit meaningful statistical comparisons within individual groups, and because the three species sampled differ from one another physically, behaviorally, and ecologically, we could not justifiably pool the groups for statistical testing of the aforementioned relationships. Spatially, positive and negative individuals were evenly distributed, although capture locations for all but five snakes were concentrated within a single 120 m stretch of rock embankment along the northwest edge of the survey site (Fig. 4). This rock embankment was constructed in 2006 and supports large local snake populations by serving as a favored basking location and source of cover. However, we suspect that this artificial structure may contribute to high ophidiomycosis prevalence in two ways. First, it encourages snakes to congregate at high densities rather than spreading more evenly across the landscape in search of natural sources of cover, and thus may accelerate horizontal transmission of *O. ophidiicola* between individuals. Second, when disturbed, snakes at this site immediately retreat downward through gaps between the rocks, spending considerable amounts of time hiding from predators beneath the embankment. This dark, damp, and muddy environment mimics the characteristics of naturally occurring snake hibernacula—habitats in which soils have been shown to contain *O. ophidiicola* at higher frequencies than surface sediment and in which the fungus is capable of proliferating even in the absence of a host (Campbell et al. 2021)—and thus may be an environmental reservoir for *O. ophidiicola*. For these reasons, future studies should investigate the role that artificial cover objects may play in increasing rates of disease transmission in snake populations affected by ophidiomycosis. When artificial refugia are intentionally deployed for research purposes, design of these objects could also take biosecurity into consideration by incorporating elements that would decrease *O. ophidiicola* habitat suitability (i.e., reduce moisture and/or increase daylight).

Our results demonstrate that *O. ophidiicola* is present in populations of at least three snake species in Rhode Island. The higher observed prevalence of the pathogen among *N. sipedon* is consistent with results from studies conducted in other states, which also note high proportions of infection within watersnake

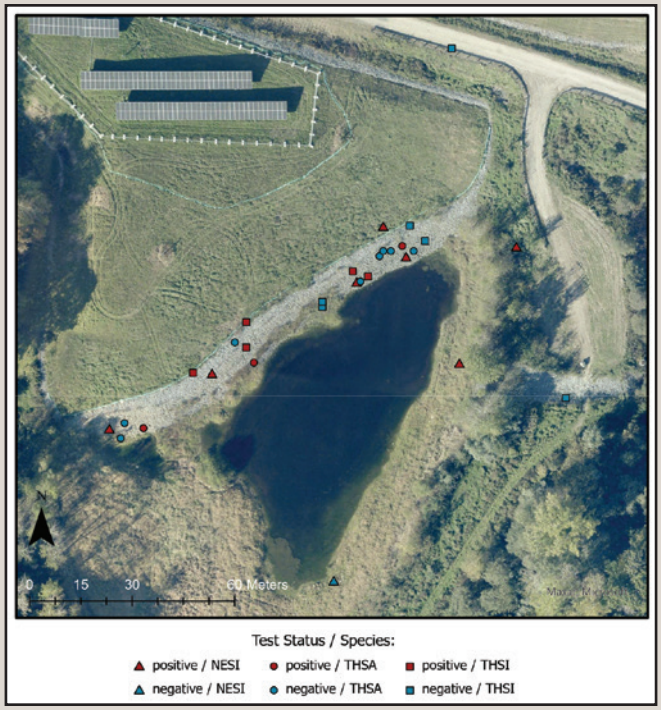

FIG. 4. Spatial distribution of snakes captured and tested for *Ophidiomyces ophidiicola* near an artificial pond in Kingston, Rhode Island, USA. Note the concentration of snakes along the manmade stone embankment at the northwest edge of the pond.

populations (Fuchs et al. 2020; Grisnik et al. 2018). However, most of the *O. ophidiicola*-positive *N. sipedon* in our study did not display any external symptoms of disease; these individuals may have been captured during the earliest stage of infection or may have been carrying the fungus externally without experiencing active ophidiomycosis. Contrasts in ophidiomycosis prevalence were also observed between *T. sirtalis* and *T. sauritus*. While nearly half of the sampled *T. sirtalis* tested positive (several of which appeared to be in poor health as evidenced by crusty brown lesions and substandard body condition), only three of 11 *T. sauritus* were found to be carrying *O. ophidiicola* and none of these individuals showed any visible sign of infection. The minimal observed impact of *O. ophidiicola* on *T. sauritus* relative to the other species we tested is encouraging, as *T. sauritus* is currently listed as a Species of Greatest Conservation Need in nine of the 14 northeastern US states within which it occurs—including Rhode Island. Although useful as initial exploratory descriptors, the interspecies comparisons of disease prevalence reported herein should be interpreted with caution due to the small sample sizes on which they are based. Further survey efforts (both planned and opportunistic) will be necessary to generate a more robust description of temporal and species-specific patterns of ophidiomycosis in Rhode Island, to determine whether the disease is present in snake populations statewide, and to assemble a list of all native species currently affected.

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