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The Occurrence of *Ophidiomyces ophiodiicola* in Northern Georgia Wild and Captive Snake Populations

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ABSTRACT: Ophidiomycosis, or snake fungal disease, is an emerging wildlife disease caused by the *Ophidiomyces ophiodiicola* fungus. The fungus can result in high mortality rates among infected snakes and has been documented across much of the eastern US, including southern Georgia. However, little is known about ophidiomycosis in northern Georgia. We surveyed wild snake populations in five counties of northern Georgia between March 2019 and March 2020 and swabbed captured snakes (n=27) for the presence of *O. ophiodiicola* DNA. We followed similar sampling protocols with a group of captive snakes (n=6) at the Elachee Nature Center in Hall County, Georgia. Quantitative PCR confirmed the presence of *O. ophiodiicola* DNA in 33% (11/33) of snakes. Eight of the confirmed positive samples were collected from wild snakes (30%, 8/27) across our sample region, while three were from our captive group (50%, 3/6). Our results indicated that *O. ophiodiicola* is present in wild snake populations in northern Georgia, and the pathogen is present in seemingly healthy captive snakes. This knowledge is critical for conservation and management efforts, but more research is needed to fully understand ophidiomycosis and its effect on snake populations in the region.

Key words: Georgia, *Ophidiomyces ophiodiicola*, ophidiomycosis, SFD, snake fungal disease, wildlife disease.

Wildlife diseases can threaten biodiversity and drive population declines across a variety of taxonomic groups (Daszak et al. 2000). For example, population declines have been documented due to chytridiomycosis in amphibians (Daszak et al. 1999), white nose syndrome in bats (Frick et al. 2010), chronic wasting disease in cervids (Rivera et al. 2019), and whirling disease in salmonid fish (Gilbert and Granath 2003). In 2006, a skin disease was discovered in a population of timber rattlesnakes (*Crotalus horridus*) in the northeastern US (Clark et al. 2011). The disease caused a population decline and was later identified as ophidiomycosis, or snake fungal disease. Ophidiomycosis often presents as ulcers or discolored and crusted scales on the head and along the body, with severe lesions most often being associated with the face (Baker et al. 2019). However, snakes can also carry the fungal pathogen on their skin asymptomatically (Thompson et al. 2018). By 2015, ophidiomycosis had been documented in wild snakes throughout most of the eastern US (Lorch et al. 2016), and substantial efforts to document the distribution and prevalence of the causative fungal pathogen in southeastern Georgia have been successful (Haynes 2020). Data from northern Georgia is lacking; therefore, we initiated sampling efforts to detect *O. ophiodiicola* among wild snake populations in northern Georgia. We also sampled a group of captive snakes to determine if any were asymptomatically carrying the fungus. We used observational data of clinical signs to contribute additional information.

We surveyed wild snake populations between March 2019 and March 2020 throughout five counties in northern Georgia: Lumpkin, Hall, Dawson, Murray, and White (Fig. 1). We conducted surveys by walking transects through public and private lands and visually searching for snakes. Upon capture, we measured snout-vent length, determined mass (g), conducted a visual inspection for skin lesions (crusted scales, discoloration, ulcerations, etc.), and determined general disposition (i.e., lethargic, active, or deceased). For snakes without visible skin lesions, we collected a single swab sample from the snake's head, as this region is most likely to show clinical signs of ophidiomycosis (Allender et al. 2011) and harbor the fungus. We gently applied firm pressure with the swab

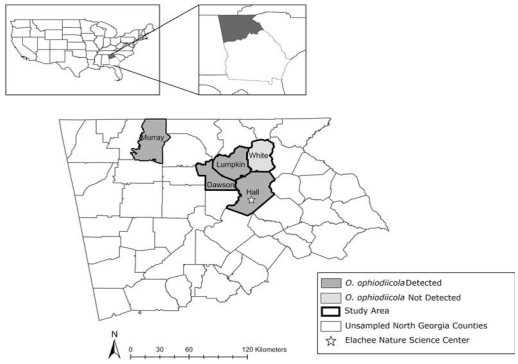

FIGURE 1. Georgia, USA, counties where we surveyed wild snake populations for *Ophidiomyces ophiodiicola*, including the location at which we tested captive snakes (Elachee Nature Center, Hall County, Georgia, USA).

while rubbing the labial scales, nostrils, eyes, and jaw using sterile handling procedures. If an individual had skin lesions resembling those of ophidiomycosis, we collected an additional swab directly from the affected areas. All snakes were released near the point of capture immediately after sample collection. We placed all swab samples in dry, 2.0-mL Eppendorf tubes stored on ice in coolers in the field and then frozen at –20 C until shipping for analysis. We also sampled a group of captive snakes at Elachee Nature Center in Hall County, Georgia using the same protocol. All captive snakes were held in individual enclosures and did not come in contact with other snakes. The frozen samples were shipped overnight on ice in a cooler and analyzed by the University of Illinois Veterinary Diagnostic Laboratory, College of Veterinary Medicine using DNA extraction and quantitative PCR amplification to detect the number of copies of *O. ophiodiicola* per ng total of DNA (copies/ng DNA).

We collected 35 swab samples from 27 wild snakes of eight species (Table 1). Five of the 27 snakes had lesions consistent with ophidiomycosis. Eight individuals, of five species, tested positive for the presence of *O. ophiodiicola* DNA (30%). One individual, a gray rat

FIGURE 2. Skin lesions including edema on the face, dermal crustiness, and missing scales on a wild *Pantherophis spiloides* (gray rat snake) that tested positive for *Ophidiomyces ophiodiicola*, with a high fungal load of 70 copies of DNA/ng based on quantitative PCR amplification.

TABLE 1. *Ophidiomyces ophiodiicola* presence in wild snake populations in northern counties of Georgia, USA, with copies of *O. ophiodiicola* DNA/ng reported. Snakes positive for *O. ophiodiicola* are in bold.

Species	Scientific name	Sample month	County	Lesions present	Copies of DNA/ng[a]
Gray rat snake	***Pantherophis spiloides***	March	Murray	Yes	Swab 1: 22.61; Swab 2: 70.16[b]
Northern black racer	*Coluber constrictor*	April	White	No	—
Garter snake	*Thamnophis sirtalis*	April	White	No	—
Brown snake	*Storeria dekayi*	April	Hall	No	—
Black rat snake	*P. obsoletus*	April	Hall	No	—
Garter snake	***T. sirtalis***	April	Hall	Yes	0.2
Ringneck snake	*Diadophis punctatus*	April	Lumpkin	No	—
Garter snake	*T. sirtalis*	May	Lumpkin	No	—
Ringneck snake	*D. punctatus*	May	Hall	No	—
Black rat snake	***P. obsoletus***	May	Hall	Yes	1.46
Black rat snake	***P. obsoletus***	May	Hall	Yes	3.47
Northern black acer	***C. constrictor***	May	Dawson	Yes	1.63
Black rat snake	*P. obsoletus*	May	Dawson	No	—
Black rat snake	*P. obsoletus*	June	Lumpkin	No	—
Black rat snake	*P. obsoletus*	September	White	No	—
Ringneck snake	*D. punctatus*	September	Lumpkin	No	—
Ringneck snake	*D. punctatus*	September	Lumpkin	No	—
Eastern worm snake	***Carphophis amoenus***	September	Lumpkin	No	10.58
Northern black racer	***C. constrictor***	September	Lumpkin	No	2.35
Eastern worm snake	*C. amoenus*	September	Lumpkin	No	—
Black rat snake	***P. obsoletus***	September	Lumpkin	No	9.42
Eastern worm snake	*C. amoenus*	September	Lumpkin	No	—
Northern water snake	*Nerodia sipedon*	October	Lumpkin	No	—
Ringneck snake	*D. punctatus*	October	Hall	No	—
Brown snake	*Storeria dekayi*	October	Hall	No	—
Ringneck snake	*D. punctatus*	October	Hall	No	—
Eastern worm snake	*C. amoenus*	October	Hall	No	—

[a] — = not applicable.

[b] This snake had two "positive" swabs in two different locations.

snake (*Pantherophis spiloides*) that exhibited lesions (Fig. 2), was swabbed upon capture and then moved into captivity. The snake tested positive with a high fungal load (70 copies/ng DNA) and died approximately 1 mo after swabbing. There was no consistent trend in geographic distribution for positive snakes. Positive samples were collected in March (n=1), April (n=1), May (n=3), and September (n=3). The reported copy number from these wild snakes ranged from 0.2–70 copies/ng DNA (Table 1). A result above 0.0 copies/ng DNA only indicates presence of the DNA. However, a result above 0.0 copies/ng DNA with clinical signs indicates a strong association with ophidiomycosis.

We collected 31 swab samples from six captive snakes of six different species (Table 2) at the Elachee Nature Center, swabbing each snake four times through the year. Three individuals tested positive for the presence of *O. ophiodiicola* when swabbed in November 2019. No snakes exhibited signs of lethargy or skin lesions. Snakes that were positive for *O. ophiodiicola* were swabbed again in January and February 2020 and results were negative. The copy number for all positive samples ranged from 0.8–5 copies/ng DNA.

Our results confirmed the presence of *O. ophiodiicola* in several snake species and indicate that the fungal pathogen is distributed across several counties in this region.

TABLE 2. *Ophidiomyces ophiodiicola* presence in Elachee Nature Center (Hall County, Georgia, USA) captive snakes with copies of *O. ophiodiicola* DNA/ng reported. Snakes positive for *O. ophiodiicola* are in bold.

Species	Scientific name	Sampling date	Sampling event	Copies DNA/ng
Black rat snake	*Pantherophis obsoletus*	April 2019	1	0.0
Corn snake	*Pantherophis guttatus*	April 2019	1	0.0
Yellow rat snake	*Pantherophis alleghaniensis*	April 2019	1	0.0
Black rat snake	*P. obsoletus*	November 2019	2	0.0
Corn snake	*P. guttatus*	November 2019	2	0.0
California king snake	***Lampropeltis californiae***	November 2019	1	4.59
Eastern king snake	***Lampropeltis getula***	November 2019	1	0.92
Corn snake	***P. guttatus***	November 2019	1	0.8
Corn snake	*P. guttatus*	January 2020	2	0.0
California king snake	*L. californiae*	January 2020	2	0.0
Eastern king snake	*L. getula*	January 2020	2	0.0
Corn snake	*P. guttatus*	January 2020	2	0.0
California king snake	*L. californiae*	February 2020	3	0.0
Eastern king snake	*L. getula*	February 2020	3	0.0
Corn snake	*P. guttatus*	February 2020	3	0.0

Previously, *O. ophiodiicola* has only been reported in southern Georgia (Haynes et al. 2020); our results bridged the gap in data coverage across the state and suggested widespread occurrence of the fungal pathogen. Although *O. ophiodiicola* was not detected in samples from White County, additional sampling may confirm its presence in most, if not all, counties in northern Georgia. The normal behavior exhibited by all sampled snakes upon capture, and apparent elimination of *O. ophiodiicola* in captive snakes, was encouraging. However, more data are needed to determine the factors contributing to the spread of *O. ophiodiicola* and to estimate the detrimental effects it could have on snake populations in northern Georgia.

The presence of *O. ophiodiicola* was confirmed in multiple individuals with no clinical signs, similar to other studies (McKenzie et al. 2019). We found that 38% (3/8) of wild individuals and 100% (6/6) of captive individuals testing positive for copies of the pathogen were asymptomatic. This suggested that the prevalence and distribution of the pathogen in Georgia may be underreported if only symptomatic individuals with obvious lesions are sampled. Lorch et al. (2015) noted behavioral changes of increased basking and increased molting by infected individuals in a laboratory setting. More data are required to understand if behavioral signs of infection manifest before external clinical signs and if infected individuals without typical skin lesions are more likely to be sampled than are uninfected individuals because of their altered behaviors. Continued sampling in northern Georgia is needed to document the distribution of the fungal pathogen in the region, host snake species, factors that contribute to the spread and impact of ophidiomycosis, and management options to mitigate any negative effects associated with the disease.

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