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Surveying for *Ophidiomyces ophidiicola* , the causal agent of Snake Fungal Disease in South Dakota.

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Abstract

For the past decade there has been an emerging disease plaguing wild snakes across the Eastern United States and Europe. In 2006, researchers started investigating the decline of Timber rattlesnake populations in New Hampshire. They discovered a fungal infection killing off the young to mid-juvenal snakes, thus know as Snake Fungal Disease or Ophidiomycosis. In 2011, San Deigo State university, identified the pathogen that causes infection, the fungus *Ophidiomyces ophiodiicola*. *O. ophiodiicola* has now affected 30 different snakes from six families within at least 20 different states since it's discovery. This study pertains to determining the prevalence of Snake Fungal Disease within South Dakota.

Introduction

In 2008, endangered snake species were also found to be affected similarly in Illinois and later in Minnesota (Lorch, et al 2016). This has massive effects on the local populations, i.e., it can kill off most local populations of snakes inhabiting closed environmental areas. The massasauga *(Sistrurus catenus)* is an endangered snake found in Minnesota/Michigan and is in the host range for Snake Fungal Disease, the presence of the fungus impedes its likelihood of a successful recovery. *Ophidiomyces ophiodiicola* can cause some forms of skin lesions (Figure 1) and dis-configurations in snakes. The term Ophidiomycosis is used only when directly addressing the infection caused by *Ophidiomyces ophiodiicola*. Red corn snakes (*Pantherophis guttatus*) infected directly using a pure culture of *O. ophiodiicola* showed the direct link to this fungus causing the ailment through direct contact with the species, causing the development of SFD. (Lorch, et al 2015). When comparing the topsoil and the soil down in hibernaculum (snake dens or pits) (Figure 4) there was a greater density of *O. ophidiicola* inside the dens than in the topsoil (Cambell, et al 2021). *O. ophiodiicola* does proliferate around the den, but that proliferation was greater in the samples taken from within the hibernacula (Campbell, et al 2021). This would suggest that the increased growth of *O. ophiodiicola* would be linked to soil pH and the composition of local microbial communities. Leading to an increase in the infectivity of the entire den due to the fungus being keratinophilic, fungus proliferates due to the abundant food that the shed provides.

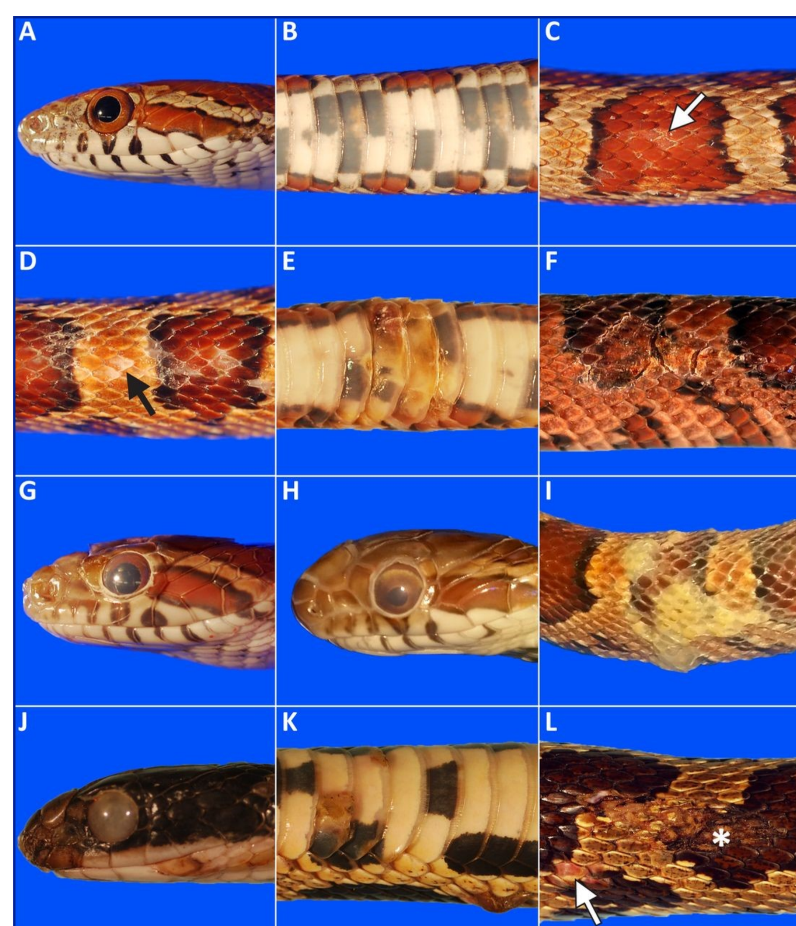

Figure 1: Lorch, et al, 2015 record of skin lesions.

Figure 4: Snake Hibernaculum

Methodology

Live Sample Collection: Snakes were found in promising areas throughout the eastern side of South Dakota with localized access to water. The snakes were swabbed on site (Figure 2). The same location wasn't used twice after samples were collected. Sabourauds agar, a growth media for fungi, supplemented with the antibiotics chloramphenicol and gentamicin plates were used to get spore samples to grow from the live swab samples. The plates were incubated at 24 °C. The Sabouraud agar plates were subcultured to make pure cultures (Figure 3). The DNA will be extracted from these pure cultures. The DNA will be PCR amplified and Sanger sequenced to determine the fungal species.

Soil Collection: These samples were collected form different counties in a mixture of deep soil samples along with mid-line den samples were collected form Lake, Codington, and Deuel Counties.

Samples were about 1.5 feet down into the ground. Samples were taken back to the lab and prepped for DNA extraction. DNA was extracted from 500 mg of each soil sample with the FastDNA Spin Kit for Soil according to the protocol. qPCR using the primer specific probes (Table 1) to test for *O. ophiodiicola*

We can identify if it is present and quantify the amount of spores within each sample.

Figure 2: A small group of snakes swabbed in Lake County

Figure 3: The isolated fungal colonies

Table 1: Species specific primers and probes

Oo-rt-ITS-F (forward primer)	5'-GAGTGATGGAATCTGTTTC-3'
Oo-rt-ITS-R (reverse primer)	5'-GGTCAAACCGGAAAGAATG-3'
Oo-rt-ITS-P (probe)	5'-(FAM)TCTCGCTCGAAGACCCGATCG(BHQ-1)-3'
Oo-rt-IGS-F (forward primer)	5'-CGGGTGAATTACCCAGTT-3'
Oo-rt-IGS-R (reverse primer)	5'-AGCCATCCTTCCCTACAT-3'
Oo-rt-IGS-P (probe)	5'-(FAM)ATACTCTCCGGGCGCTTGTCTTCC(BHQ-1)-3'

Results

- Pure Cultures obtained from Live Sampling
- qPCR protocol designed
- Samples Awaiting testing

Future Directions

- Extract DNA from Culture
- Run qPCR soil, culture, and shed
- Ecological Survey

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