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-juvenile snakes, thus know as Snake Fungal Disease or Ophidiomycosis. In 2011, San Diego State university, identified the pathogen that causes infection, the fungus *Ophidiomyces ophidiicola*. *O. ophidiicola* 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.

**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. Sabouraud 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 from 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 prepared 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. ophidiicola*

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

**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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**Table 1:** Species specific primers and probes

<table>
<thead>
<tr>
<th>Oo-r-ITS-F (forward primer)</th>
<th>5'-GAGTTGATGGAACATGTTTCTG-3'</th>
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<tbody>
<tr>
<td>Oo-r-ITS-R (reverse primer)</td>
<td>5'-GGTCACCCGAAAAGAAGTG-3'</td>
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<tr>
<td>Oo-r-ITS-P (probe)</td>
<td>5'-GAGTTGATGGAACATGTTTCTG-3'</td>
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<tr>
<td>Oo-r-RS-F (forward primer)</td>
<td>5'-CGGAGAATTTCTCCACTAT-3'</td>
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<tr>
<td>Oo-r-RS-R (reverse primer)</td>
<td>5'-ACCACCTTACCTTACGAT-3'</td>
</tr>
<tr>
<td>Oo-r-RS-P (probe)</td>
<td>5'-GAGTTGATGGAACATGTTTCTG-3'</td>
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