2017 Snake Fungal Disease Monitoring Report For Tennessee

Josh Campbell, Wildlife Diversity Coordinator

Tennessee Wildlife Resources Agency

Region 2

TWRA Wildlife Technical Report 17-4

Snake Fungal Disease Monitoring Report

For Tennessee

Photo Credit: Danny Bryan. A timber rattlesnake showing clinical symptoms of snake fungal disease.

Acknowledgements

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A tremendous amount of work has been conducted by partners and collaborators increasing our knowledge regarding snake fungal disease in Tennessee. Additional contribution was made by Dr. Walker in providing valuable information and review to this report.

Acronyms

CU……Cumberland University

MTSU……..……..Middle Tennessee State University

UTK.....University of Tennessee, Knoxville

TTU……...……...……..Tennessee Technological University

TWRA.....Tennessee Wildlife Resources Agency

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Executive Summary

Snake Fungal Disease (SFD) is an emerging skin disease affecting both free ranging and captive snake species across North America. SFD can be extremely debilitating to snakes, and in severe cases, lethal or requiring human intervention and euthanasia. In 2009, the fungus *Chrysosporium ophiodiicola* was described from a black rat snake (*Elaphe obsoleta obsoleta*) in Georgia. This individual presented symptoms of large, slow-growing facial masses. The fungus was later recircumscribed as *Ophidiomyces ophiodiicola* in the family *Onygenaceae* based on morphological, cultural and molecular data. The majority of disease cases reported have been restricted to the eastern United States. SFD has been confirmed in 20 U.S. states (Florida, Georgia, New Hampshire, Illinois, Massachusetts, Michigan, Louisiana, Virginia, Pennsylvania, South Carolina, Wisconsin, Kentucky, Ohio, New Jersey, New York, Alabama, Minnesota, Connecticut, Vermont, and Tennessee) and Canada. SFD has been documented in 30 species of snakes in North America and it is possible impacts may vary by species, population, and geographically. Reports and observations of SFD have increased across the eastern United States in recent years.

SFD was first confirmed in Tennessee during fall of 2012 in samples taken from a timber rattlesnake (*Crotalus horridus*) collected in Dekalb County. During the following spring, additional samples collected from both a timber rattlesnake and queen snake (*Regina septemviattta*) collected in Rutherford County, TN also tested positive. It is highly likely SFD may have been present in some populations prior to these confirmations as researchers have observed snakes in other portions of the state in previous years with clinical symptoms similar to those presented above. However, these early reports of snake fungal disease in Tennessee are speculation. Since the project was initially implemented, over 235 samples have been collected from 20 snake species in the state. Samples have been collected in 27 counties and *O. ophiodiicola* was confirmed in or present on 14 snake species across 16 Tennessee counties

Table of Contents

List of Figures

List of Tables

Introduction

The occurrence of virulent infectious diseases in natural wildlife populations has increased in recent years, leading to significant animal die-offs and extinctions (Fisher et al. 2012). Adequately addressing these outbreaks by state and federal agencies and conservationists has become increasingly difficult. Snake Fungal Disease (SFD) is an emerging skin disease affecting both free ranging and captive snake species across North America. SFD can be extremely debilitating to snakes, and in severe cases, lethal or requiring human intervention and euthanasia. The majority of disease cases reported have been restricted to the eastern United States (Figure 1). SFD has been confirmed in 20 U.S. states (Florida, Georgia, New Hampshire, Illinois, Massachusetts, Michigan, Louisiana, Virginia, Pennsylvania, South Carolina, Wisconsin, Kentucky, Ohio, New Jersey, New York, Alabama, Minnesota, Connecticut, Vermont, and Tennessee) and Canada (Cheatwood et al. 2003; Rajeev et al. 2009; Clark et al. 2011; Allender et al. 2011; Allender et al. 2013; SCDNR 2013; Dolinski et al. 2014; Allender et al. 2015; Fenton et al. 2015; McBride et al. 2015; Price et al. 2015; Tetzlaff et al. 2015; Allender et al. 2016; Glorioso et al. 2016; Guthrie et al. 2016; Last et al. 2016; Lorch et al. 2016; NWHC 2016; Ohkura et al. 2016; Ravesi et al. 2016; WDNR 2016; Stephen et al. 2017). SFD has been documented in 30 species of snakes in North America (Table 1) and it is possible impacts may vary by species, population, and geographically. Reports and observations of SFD have increased across the eastern United States in recent years. It should be noted, research on SFD is extremely difficult under natural conditions given the secretive nature and behavior of snakes making follow up observations difficult to obtain, greatly decreasing our knowledge regarding this wildlife epidemic.

History

In 2009, the fungus *Chrysosporium ophiodiicola* was described from a black rat snake (*Elaphe obsoleta obsoleta*) in Georgia. This individual presented symptoms of large, slow-growing facial masses (Rajeev et al. 2009). The fungus was later recircumscribed as *Ophidiomyces ophiodiicola* in the family *Onygenaceae* based on morphological, cultural and molecular data (Sigler et al. 2013). Species in this family are a group of fungi commonly associated with disease in reptiles. There are nine different pathogenic fungal species in the *Onygenaceae* which affect various reptiles (Schmidt 2015)

After the identification of the fungus *O. ophiodiicola* on snake skin, researchers sought to determine how the fungus and snake fungal disease were linked. Lorch et al. (2015), through laboratory experimentation with captive-bred corn snakes (*Pantherophis guttatus*), established a direct causal link between *O. ophiodiicola* and SFD, although the presence of other fungal species is common. Furthermore, Lorch et al. (2015) were able to document the progression of the disease in snakes and identify their response to infection lending explanation to the variation of the disease in reported observations. Allender et al (2015b) developed a qPCR assay to rapidly quantify fungal load of *O. ophiodiicola* thus aiding in conservation of snakes through identification of emerging and ongoing outbreaks of SFD. Further PCR testing conducted by Bohuski et al. (2015) identified *O. ophiodiicola* in the absence of clinical symptoms of SFD, increasing its use as a diagnostic tool.

Figure 1. Confirmation of SFD by state through 2016.

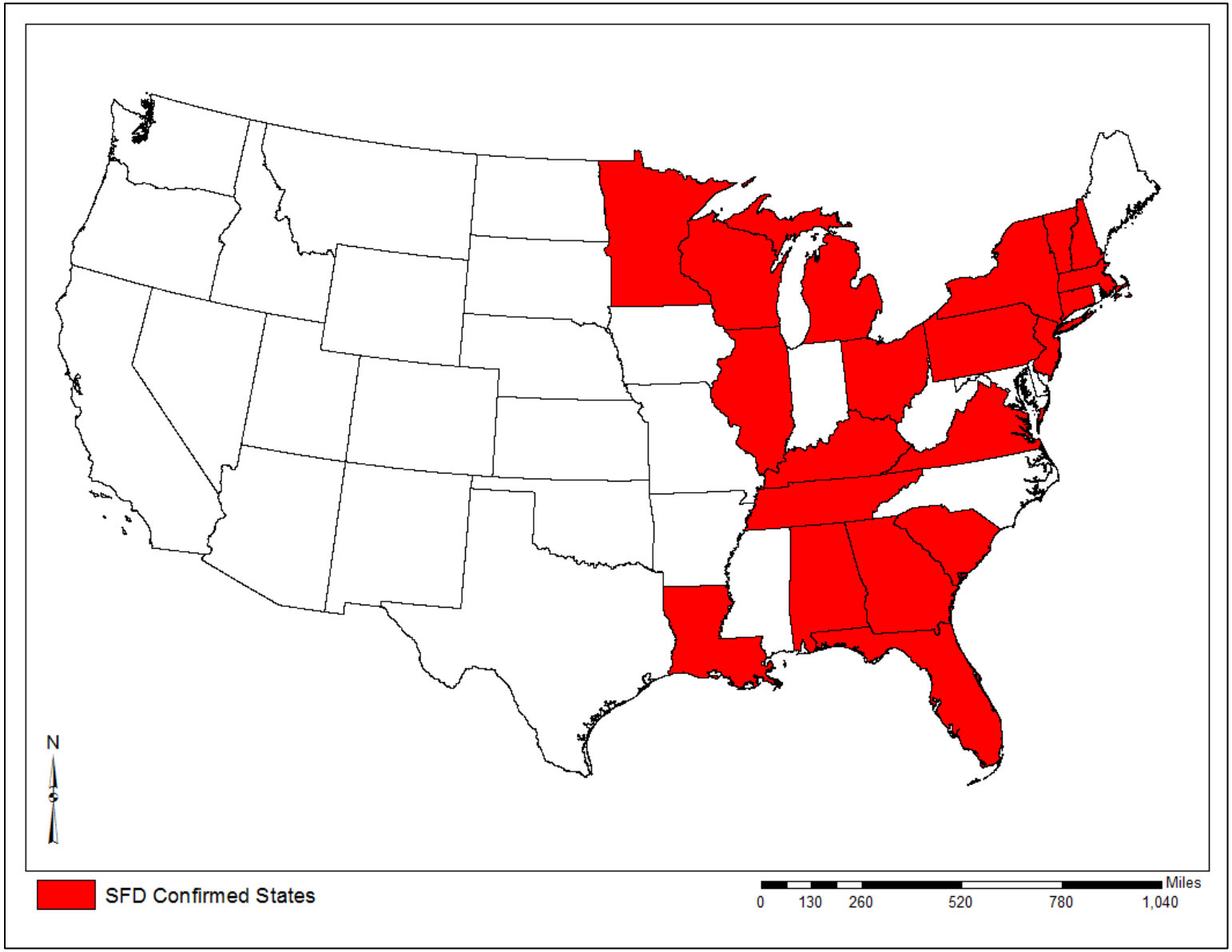


Table 1. Snake species that have tested positive for *O. ophiodiicola*.

Species	Year	Location	Origin	References
Acrochordidae				
Java Wart Snake, *Acrochordus arafurae*	2003	Austrailia	Captive	Sigler et al. 2013
Pythonidae				
Ball Python, *Python regius*	1985	England	Captive	Sigler et al. 2013
African Rock Python, *Python sebae*	2001	NM	Captive	Sigler et al. 2013
Boiidae				
Green Anaconda, *Eunectes murinus murinus*	2008	CA	Captive	Sigler et al. 2013
Colubridae				
Corn Snake, *Elaphe guttata guttata*	1986	N/A	N/A	Sigler et al. 2013
Brown Tree Snake, *Boiga irregularis*	1990	Guam	Captive	Nichols et al. 1999
Garter Snake, *Thamnophis sp.*	1999	Germany	Captive	Sigler et al. 2013
Salt March Snake, *Nerodia clarkii*	2006	FL	Captive*	Sigler et al. 2013
Black Rat Snake, *E. obsoleta obsoleta*	2009	GA	Captive*	Rajeev et al. 2009
Plains Garter Snake, *T. radix*	2012	IL	Wild	Dolinski et al. 2014
Queen Snake, *Regina septemvittata*	2013	TN	Wild	SCWDS: CC13-161
Brown Water Snake, *N. taxispilota*	2014	VA	Wild	Guthrie et al. 2016
Eastern Black Racer, *Coluber constrictor*	2014	VA	Wild	Guthrie et al. 2016
Eastern Black Racer, *C. constrictor*	2014	TN	Wild	SCWDS: CC14-139
Eastern Milksnake, *Lampropeltis triangulum*	2014	TN	Wild	SCWDS: CC14-139
Eastern Rat Snake, *Pantherophis alleghaniensis*	2014	VA	Wild	Guthrie et al. 2016
Mud Snake, *Farancia abacura*	2014	GA	Wild	Fenton et al. 2015; Last et al. 2016
Norther Water Snake, *N. sipedon*	2014	VA	Wild	Guthrie et al. 2016
Queen Snake, *R. septemvittata*	2014	KY	Wild	Price et al. 2015
Rainbow Snake, *F. erytrogramma*	2014	VA	Wild	Guthrie et al. 2016
Broad-banded Water Snake, *N. fasciata confluens*	2015	LA	Wild	Glorioso et al. 2016
Eastern Milksnake, *L. triangulum*	2015	MI	Wild	Ravesi et al. 2016
Eastern Fox Snake, *P. vulpina*	2014-2015	WI	Wild	WDNR 2016
Milksnake, *L. triangulum*	2014-2015	WI	Wild	WDNR 2016
Common Kingsnake, L. getula	2016	TN	Wild	Walker and Leys 2017
Garter Snake, *T. sirtalis*	2016	TN	Wild	Walker and Leys 2017
Norther Water Snake, *N. sipedon*	2016	TN	Wild	Walker and Leys 2017
Ribbon Snake, *T. sauritus*	2016	TN	Wild	Walker and Leys 2017

Species	Year	Location	Origin	References
Western Rat Snake, *P. obsoletus*	2016	TN	Wild	Walker and Leys 2017
Black Kingsnake, *L. nigra*	N/A	TN	Wild	M. Allender, personal communication, February 15, 2017
Eastern Black Racer, *C. constrictor*	N/A	PA	Captive	Ohkura et al. 2016
Eastern Black Racer, *C. constrictor*	N/A	TN	Wild	M. Allender, personal communication, February 15, 2017
Eastern Fox Snake, *P. vulpina*	N/A	N/A	N/A	Stephen et al. 2017
Garter Snake, *T. sirtalis*	N/A	PA	Captive*	Ohkura et al. 2016
Garter Snake, *T. sirtalis*	N/A	N/A	N/A	Stephen et al. 2017
Queen Snake, *R. septemvittata*	N/A	N/A	N/A	Stephen et al. 2017
Bullsnake, *Pituophis catenifer sayi*			Wild	Lorch et al. 2016
Louisiana Pinesnake, *Pituuophis ruthveni*			Wild	Lorch et al. 2016
Smooth Earthsnake, *Virginia valeriae*			Wild	Lorch et al. 2016
Western Ribbon Snake, *T. proximus*			Wild	Lorch et al. 2016
Eastern Hognose Snake, *Heterodon platirhinos*	2017	TN	Wild	USGS 28376
Eastern Milksnake, *L. triangulum*	2017	TN	Wild	USGS 28376
Black Kingsnake, *L. nigra*	2017	TN	Wild	USGS 28376
Eastern Milksnake, *L. triangulum*	2017	TN	Wild	USGS 28376
Eastern Black Racer, *C. constrictor*	2017	TN	Wild	USGS 28376
Corn Snake, *P. guttatus*	2017	TN	Wild	USGS 28376
Eastern Black Racer, *C. constrictor*	2017	TN	Wild	USGS 28376
Western Rat Snake, *P. obsoletus*	2017	TN	Wild	USGS 28376
Eastern Black Racer, *C. constrictor*	2017	TN	Wild	USGS 28376
Viperidae				
Pigmy Rattlesnake, *Sistrurus miliarius barbouri*	1997-1998	FL	Wild	Cheatwood et al. 2003
Eastern Diamondback Rattlesnake, *Crotalus adamanteus*	2006	FL	N/A	Sigler et al. 2013
Timber Ratlesnake, *Cr. horridus*	2006	NH	Wild	Clark et al. 2011
Eastern Massasauga Rattlesnakes, *S. catenatus catenatus*	2008	IL	Wild	Allender et al. 2011
Eastern Massasauga Rattlesnakes, *S. catenatus catenatus*	2011	IL	Wild	Allender et al. 2013
Timber Ratlesnake, *Cr. horridus*	2012	TN	Wild	NWHC-24216
Timber Ratlesnake, *Cr. horridus*	2012	MN	Wild	Smith et al. 2013
Copperhead, *Agkistrodon contortrix*	2013	SC	Wild	SCDNR 2013
Massasauga Rattlesnakes, *S. catenatus*	2013	MI	Wild	Tetzlaff et al. 2015
Timber Ratlesnake, *Cr. horridus*	2013	TN	Wild	SCWDS: CC13-161
Timber Ratlesnake, *Cr. horridus*	2011-2013	MA	Wild	McBride et al. 2015
Massasauga Rattlesnakes, *S. catenatus*	2014	MI	Wild	Allender et al. 2016

Species	Year	Location	Origin	References
Timber Ratlesnake, *Cr. horridus*	2014	TN	Wild	SCWDS: CC14-49
Timber Ratlesnake, *Cr. horridus*	2014	TN	Wild	SCWDS: CC14-139
Timber Ratlesnake, *Cr. horridus*	2014-2015	WI	Wild	WDNR 2016
Timber Ratlesnake, *Cr. horridus*	2016	TN	Wild	SCWDS: CC16-281
Timber Ratlesnake, *Cr. horridus*	2016	TN	Wild	SCWDS: CC16-362
Cottonmouths, *A. piscivorous*	N/A	IL	Captive*	Allender et al. 2015
Eastern Massasauga Rattlesnakes, *S. catenatus catenatus*	N/A	N/A	N/A	Stephen et al. 2017
Timber Ratlesnake, *Cr. horridus*	2017	TN	Wild	USGS 28376
Elapidae				
Broad-headed Snake, *Hoplocephalus bungaroides*	2010	Austrailia	Captive	Sigler et al. 2013

* Originally collected in the wild and maintained in captive situation.

Clinical Symptoms

Reports and observations of SFD vary tremendously based on the stage of infection at the time of report, observation, or capture of the animal (Appendices A-E). Snakes presenting with SFD may be observed with ulcerated and/or eroded skin, incomplete sheds, large nodules on the head or other parts of the body, severely malformed heads, appear malnourished or lethargic, and unusual behaviors, to include frequently observed in the open or basking at unusual times. *O. ophiodiicola* invades the superficial skin of snakes causing lesions. These lesions swell and lead to thickening, crusting, and eventual death of the epidermis. During molting, the shedding skin bunches up, with portions adhering to one another, leading to dysecdysis (portions of the molt being retained on the new skin). After this, the crust falls off the snake, the ulcerated and eroded skin is revealed. Some scales may be smaller, deformed or depigmented as a result of these lesions. Snakes also develop dermal granulomas on the head, neck, and chin. The effects of lesions developed as the result of SFD can vary, be mild to severe or ultimately lethal. In addition, this fungus has been routinely detected in asymptomatic snakes leading researchers to question if these snakes haven't yet developed symptoms characteristic of SFD, or alternatively if *O. ophiodiicola* occurs as a commensal member of the snake microbiome.

Epidemiology

O. ophiodiicola infections begin when the outermost layer of the skin is compromised allowing the fungus to enter the epidermis. Following invasion of the fungus into the snake's skin, the immune response includes swelling and buildup of fluid around infected skin tissues. Within days of the infection, the characteristic yellow to brown crusts form and the skin becomes necrotic and thickened. Skin erosion or ulcers are caused from the breaking off of these crusts. As the fungal growth continues within the necrotic skin, lesions may continue to expand in size.

Figure 2. SFD leads to the development of a yellow to brown crust on the scales.

Danny Bryan

Most *O. ophiodiicola* infections are limited to the epidermis, but occasionally fungal hyphae may penetrate the dermis. In severe infections, the fungus may invade deep skin layers and skeletal muscle. With these infections, the fungus is often encased within granulomas that present as nodules. Fungal invasions of the cornea, maxillary bone, and lungs have been reported, but these reports are not widespread in wild populations as it is believed most snakes succumb to secondary diseases prior to infections reaching these advanced states.

Lorch et al. (2015) reported increased molt frequency in captive snakes infected with *O. ophiodiicola*. During molt, the necrotic and diseased epidermis are removed, and the new skin appears normal, although some scales may appear deformed or reduced in size. In most cases, molting appears to clear the fungus and snakes may recover. However, in the event the fungal

invasions are deep into the epidermis or the fungus invades the new skin prior to molting, snake fungal disease may reoccur. Multiple molts with increased frequency may be required to clear infections.

Danny Bryan

Figure 3. Sever SFD infections can lead to ocular occlusions.

Mortality associated with disseminated *O. ophiodiicola* infections are likely the result of complications of the infection rather than direct fungal damage (Lorch et al. 2016). Infections of the head which impact vision, olfaction and infrared sensing likely impact the ability to search and capture prey. Observations of wild snakes often report emaciation. As *O. ophiodiicola* infections persist, the overall health of a snake declines, increasing the likelihood of secondary infections or diseases.

Innate Immunity Correlates with *Ophidiomyces ophiodiicola*

The idea of microbiome (resident skin microflora) mediated pathogen resistance is not a unique concept; microbiome function as a part of the innate immune system has been documented in vertebrates ranging from a variety of mammals, including humans, to many species of amphibians (Gao et al. 2008, Grice et al. 2009, Scharschmidt et al. 2009, Bletz et al. 2013). The host microbiome serves to confer disease resistance to pathogenic fungi by producing antifungal metabolites, outcompeting the fungus for space, or by stabilizing the microbial community to drive defense efficacy (Lauer et al. 2007, Bletz et al. 2013). For example, it has been suggested that persistent symbiotic microorganisms were a key element in the survival of the mountain yellow-legged frog (*Rana muscosa)* in the presence of chytrid mycosis (Woodhams et al. 2007), and that the microbiome of European water frogs (*Pelophylax esculentus* and *P. lessonae*) was responsible for reducing the subclinical costs of infection by introduced *B. dendrobatidis* (Woodhams et al. 2012). Several studies have documented the gut microbiome of a limited number of reptilian species but bacterial members of the cutaneous microbiome remain undocumented with respect to their community (Costello et al. 2010, Keennan et al. 2013). Hill et al. (2017) isolated 23 bacterial and five fungal isolates from the skin of a Black Racer (*Coluber constrictor*) and a Timber rattlesnake (*Crotalus horridus*), and found each species maintained a unique microbiome with no overlap. In addition, seven strains of bacteria were documented to have antifungal activity when challenged against *O. ophiodiicola.* Given these results it is conceivable that the snake cutaneous microbiome plays a role in host defense during early stages of *O. ophiodiicola* infection. Walker and Leys (manuscript in prep.) determined that the snake cutaneous microbiome differs by species, geographic location, and correlates with the presence/absence of the fungal pathogen, *O. ophiodiicola* (Figures 4 - 6). These results imply that the host microbiome of snakes may act in an innate immunity response protecting the snake

from disease. Alternatively, perhaps after the snake becomes infected by the fungus the microbiome shifts as a response to infection.

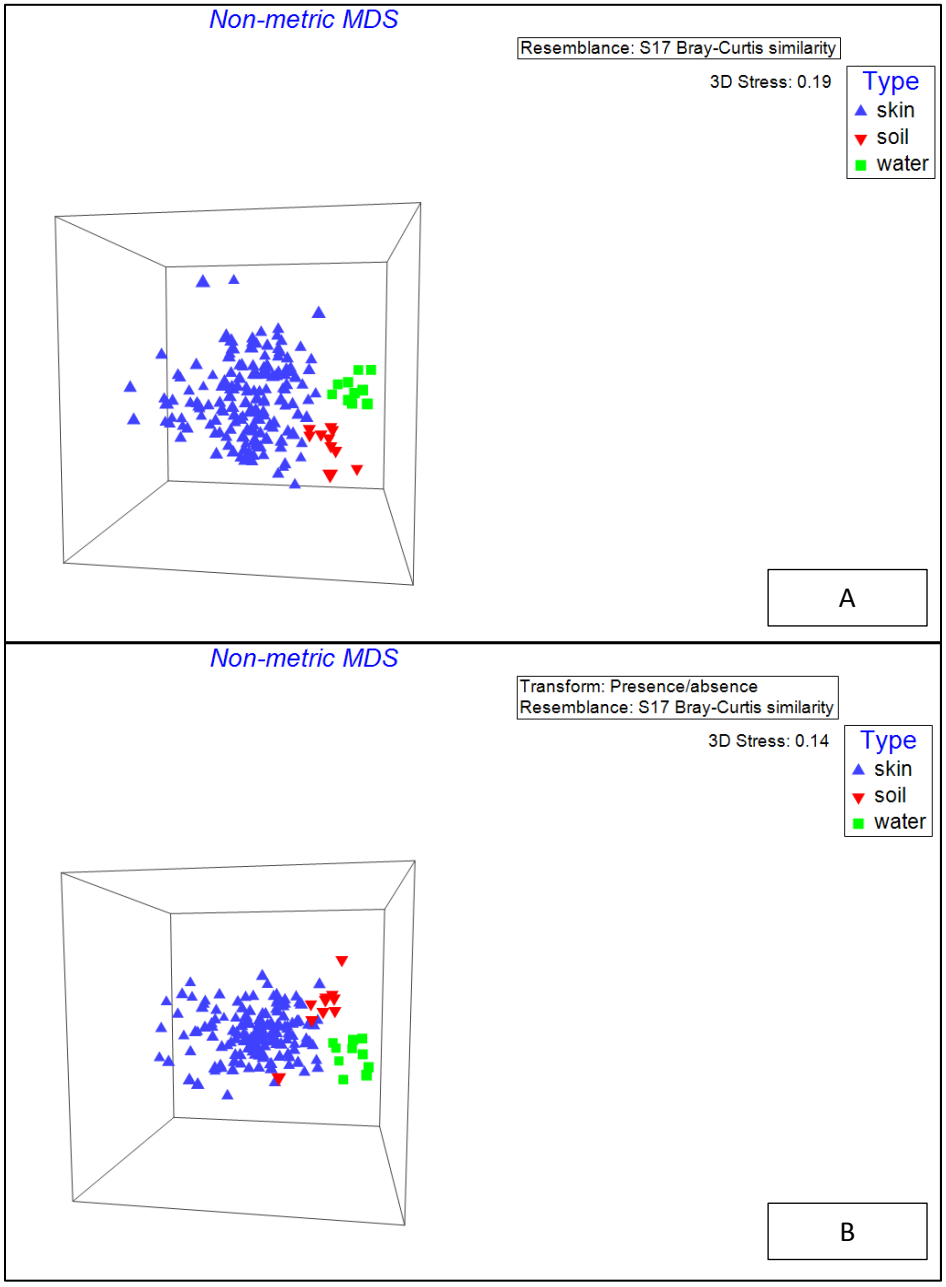


Figure 4. Beta diversity patterns of the cutaneous snake microbiome compared with environmental microbial communities from both soil and water, visualized using a non-metric multidimensional scaling ordination. A. Ordination based on relative abundance of subsampled OTUs. B. Ordination based on Jaccard (presence/absence) transformation of OTUs.

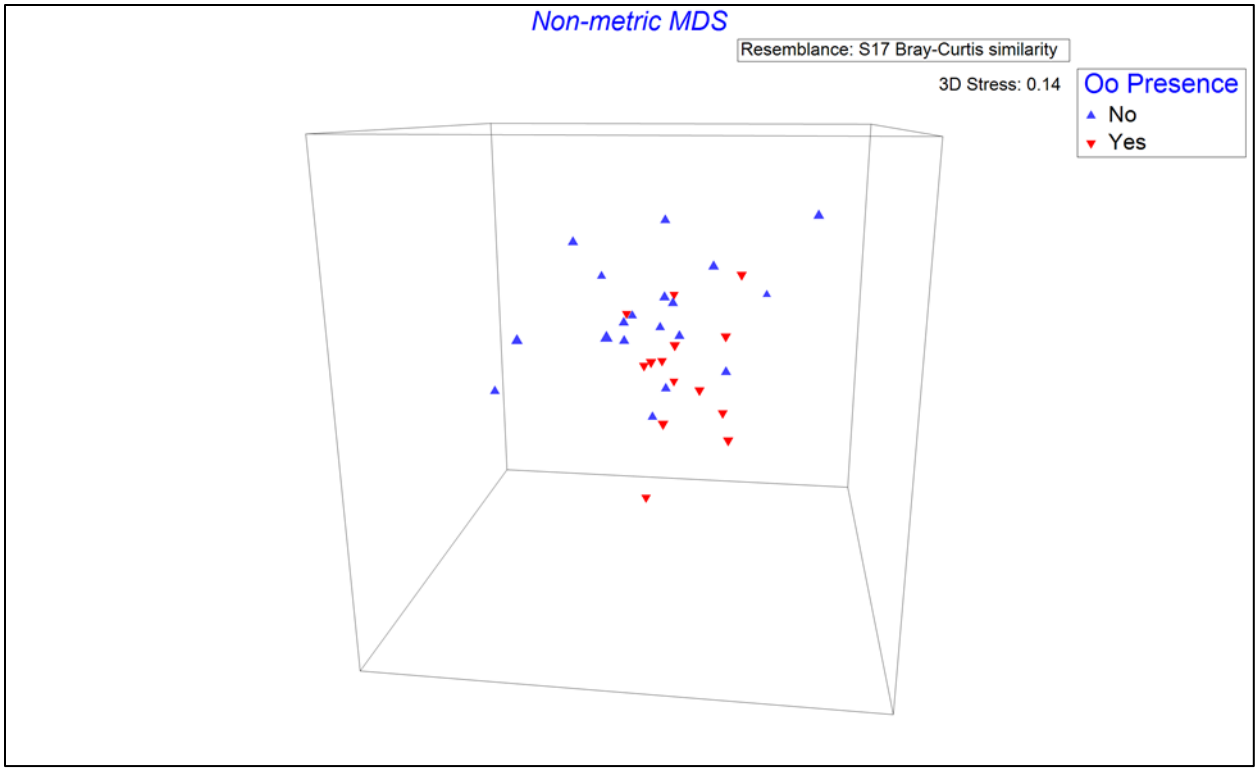


Figure 5. Beta-diversity of cutaneous samples averaged across species and visualized by non-metric multidimensional scaling. The presence/absence of *O. ophiodiicola* as determined by qPCR is plotted on each ordination.

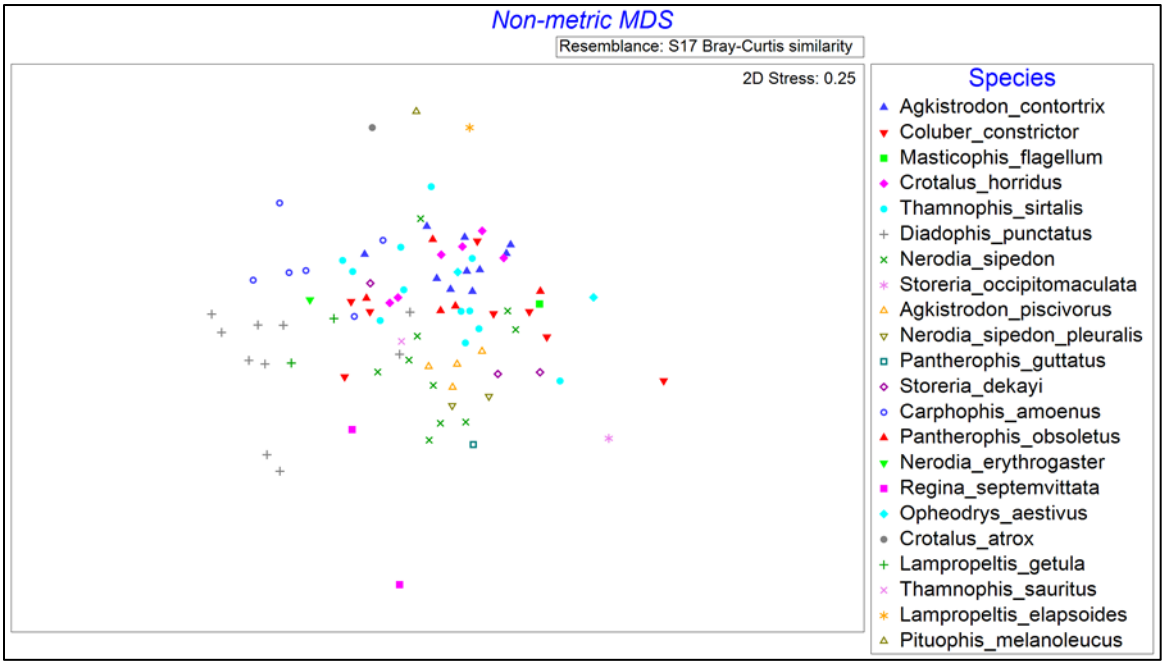


Figure 6. Beta-diversity of cutaneous samples plotted by species and visualized by non-metric multidimensional scaling.

Environmental Reservoir of *Ophidiomyces ophiodiicola*

While the fungus *O. ophiodiicola* has recently been confirmed as the causative agent of SFD through successful application of Koch's postulates (Lorch et al. 2015), there is scant information regarding the characteristics and life history of this pathogen. Recently, Allender et al. (2015c) performed a series of *in vitro* experiments with isolates of *O. ophiodiicola* cultured from infected free-ranging Massasauga rattlesnakes (*Sistrurus catenatus*) and one Plains garter snake (*Thamnophis radix*), and subsequently demonstrated the wide range of temperatures, pH, and matric induced water stress *O. ophiodiicola* can tolerate. This fungus has also been shown to utilize complex carbon, nitrogen, and sulfur resources (Allender et al. 2015c). Founded on these *in vitro* based experiments, Allender et al. (2015c) hypothesized that this fungus opportunistically infects snakes, and persists in the soil, but have yet to support this hypothesis. Preliminary evidence from Walker and Leys (manuscript in preparation) based on inoculating the fungus into the soil and observing it for growth indicated that the fungus is capable of utilizing soil as a source of nutrition. In addition, soil samples were taken from point capture locations of 40 snakes, tested using qPCR, and two cases confirmed positive for the presence of *Ophidiomyces ophiodiicola* (manuscript in preparation). The subsequent visual and molecular detection of hyphae characterized as *O. ophiodiicola* in the soil of a controlled lab experiment, in conjugation with the in vitro experiments performed by Allender et al. (2015c), create a reasonable body of evidence that suggests this fungus persists in the soil. Future work should aim to observe the fungus during sporulation within a natural environment as a final confirmation of soil as a reservoir.

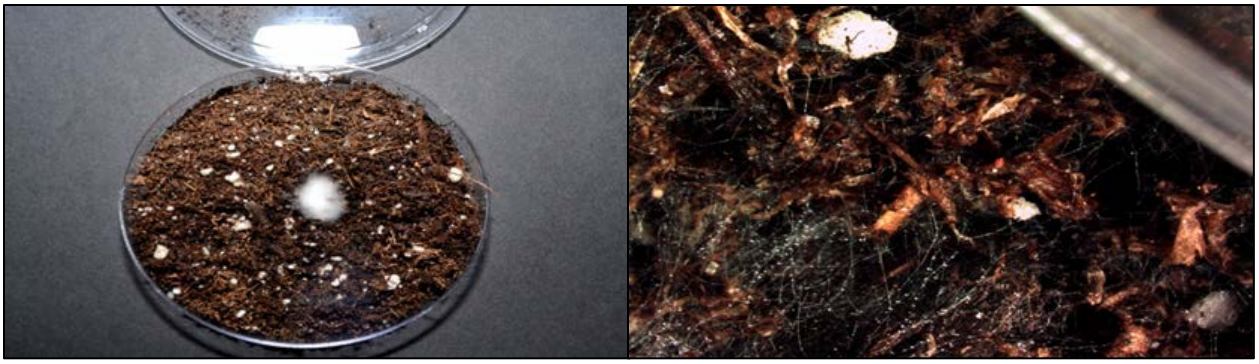
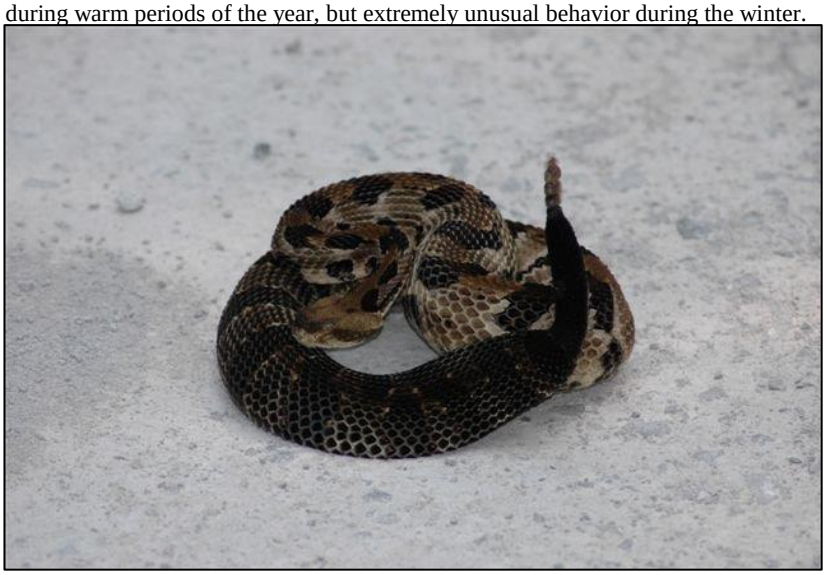


Figure 7. *Ophidiomyces ophiodiicola* growing in soil medium. The left image shows a small white fungal colony in the center of the plate. The right panel shows hyphal filaments extending to the edge of the petri plate.

Behavioral Changes

Figure 8. Encountering timber rattlesnakes in open areas, such as roads, is common during warm periods of the year, but extremely unusual behavior during the winter.



Chris Simpson

Additional forms of mortality of snakes afflicted with SFD may occur as unusual behaviors have been reported. Experimentally infected snakes were observed in conspicuous areas of containers compared to the underneath of provided shelters (Lorch et al. 2015). Snakes may be observed in the open more frequently or outside of hibernaculum during unusual periods of the year. Clark et al. 2011 noted numerous timber rattlesnakes (*Cortalus horridus*) outside of a hibernaculum in New Hampshire the first week of November when typical behavior places them in winter dens a month earlier, and it was believed this behavior was the result of infection or disease. Similar behaviors were reported by McBride et al. (2015) as multiple timber rattlesnakes were observed basking in the sun during winter in Massachusetts. While there are advantages for increased movement during the winter (increased immune response to infection, Kluger 1979), emerging from winter hibernaculum increases predation risks for snakes. For example, Nordberg and Cobb (2016) speculated at least one timber rattlesnake was predated during their study in Rutherford County, Tennessee.

In Tennessee

SFD was first confirmed in Tennessee during fall of 2012 in samples taken from a timber rattlesnake collected in Dekalb County (Appendix A). During the following spring, additional samples collected from both a timber rattlesnake and queen snake (*Regina septemviattta*) collected in Rutherford County, TN also tested positive (Appendix B). It is highly likely SFD may have been present in some populations prior to these confirmations as researchers have observed snakes in other portions of the state in previous years with clinical symptoms similar to those presented above. However, these early reports of snake fungal disease in Tennessee are speculation.

During 2013, the Tennessee Wildlife Resources Agency began participation in a multistate effort to aid conservation of snake species of greatest conservation need threatened by this emerging fungal skin disease. During this project, TWRA and researchers from MTSU, CU, and UTK collected samples from timber rattlesnakes from multiple populations to ascertain the presence of *O. ophiodiicola*, as it was the only snake species of greatest conservation within the state known to be impacted from the disease. Funding from the project was also used to

implement research projects in middle Tennessee with the goals of gaining insight into the physiologic and behavioral responses of timber rattlesnakes to *O. ophiodiicola*.

In 2016, a third project was implemented with researchers from TTU to build off the work conducted during 2013 and 2014, in which the goals were not only to continue to identify the impacts of *O. ophiodiicola* on timber rattlesnakes, but also to gain valuable insight into the geographic range of this fungal pathogen within the state. Since the project was initially implemented, over 235 samples have been collected from 20 snake species in the state. A portion of these samples are listed in Table 2. Samples have been collected in 27 counties and *O. ophiodiicola* was confirmed in or present on 14 snake species across 16 Tennessee counties (Figures 5 and 6).

Monitoring and collection of samples to determine the geographic range of *O. ophiodiicola* have continued into 2017 and are planned for 2018. Potential environmental reservoirs of *O. ophiodiicola*, to include the water and soil, have been investigated. Because *O. ophiodiicola* is a fungus, environmental co-factors will impact the severity of outbreaks within the state, meriting their investigation. A changing climate will also have an impact on the severity SFD has within the state as milder winters across regions are anticipated, the vulnerability of snakes to SFD is expected to increase (Allender et al. 2015c). Warming hibernation temperatures and wetter winters have been previously linked to declines of snake populations, particularly timber rattlesnakes (Clark et al. 2011), but the severity of outbreaks will likely effect snake populations differently across geographic scales.

Table 2. Tennessee collections and prevalence of *O. ophiodiicola* by species and collection location (Walker and Leys, 2017). Highlighted species tested positive for *O. ophiodiicola*.

Species	Scientific Name	County	*Oo* Detected
Copperhead	*Agkistrodon contortrix*	Bledsoe / Van Buren	No
Copperhead	*Agkistrodon contortrix*	Bledsoe / Van Buren	No
Common Garter Snake	*Thamnophis sirtalis*	Bledsoe / Van Buren	No
Cottonmouth	*Agkistrodon piscivorus*	Cheatham	No
Black Racer	*Coluber constrictor*	Cheatham	No
Ring-necked Snake	*Diadophis punctatus*	Cheatham	No
Common Kingsnake	*Lampropeltis getula*	Cheatham	Yes
Plain-bellied Water Snake	*Nerodia erythrogaster*	Cheatham	Yes
Northern Water Snake	*Nerodia sipedon*	Cheatham	No
Northern Water Snake	*Nerodia sipedon*	Cheatham	No
Northern Water Snake	*Nerodia sipedon*	Cheatham	No
Northern Water Snake	*Nerodia sipedon*	Cheatham	Yes
Ribbon Snake	*Thamnophis sauritus*	Cheatham	Yes
Common Garter Snake	*Thamnophis sirtalis*	Cheatham	No
Black Rat Snake	*Pantherophis obsoletus*	Cocke	No
Black Rat Snake	*Pantherophis obsoletus*	Cocke	Yes
Copperhead	*Agkistrodon contortrix*	Cumberland	No
Worm Snake	*Carphophis amoenus*	Cumberland	No
Worm Snake	*Carphophis amoenus*	Cumberland	No
Black Racer	*Coluber constrictor*	Cumberland	No
Black Racer	*Coluber constrictor*	Cumberland	No
Timber Rattlesnake	*Crotalus horridus*	Cumberland	No
Timber Rattlesnake	*Crotalus horridus*	Cumberland	No
Timber Rattlesnake	*Crotalus horridus*	Cumberland	Yes
Timber Rattlesnake	*Crotalus horridus*	Cumberland	Yes
Ring-necked Snake	*Diadophis punctatus*	Cumberland	No
Northern Water Snake	*Nerodia sipedon*	Cumberland	No
Northern Water Snake	*Nerodia sipedon*	Cumberland	No
Common Garter Snake	*Thamnophis sirtalis*	Cumberland	Yes
Queen Snake	*Regina septemvittata*	Davidson	No
Black Racer	*Coluber constrictor*	Dekalb	No
Timber Rattlesnake	*Crotalus horridus*	Dekalb	No
Timber Rattlesnake	*Crotalus horridus*	Dekalb	Yes
Timber Rattlesnake	*Crotalus horridus*	Dekalb	Yes
Rough Green Snake	*Opheodrys aestivus*	Dekalb	No
Black Rat Snake	*Pantherophis obsoletus*	Greene	Yes
Ring-necked Snake	*Diadophis punctatus*	Grundy	No
Rough Green Snake	*Opheodrys aestivus*	Grundy	No
Rough Green Snake	*Opheodrys aestivus*	Grundy	No
Worm Snake	*Carphophis amoenus*	Hamblen	No
Black Racer	*Coluber constrictor*	Jackson	No
Ring-necked Snake	*Diadophis punctatus*	Jackson	Yes
Northern Water Snake	*Nerodia sipedon*	Jackson	No
Northern Water Snake	*Nerodia sipedon*	Jackson	No
Northern Water Snake	*Nerodia sipedon*	Jackson	Yes
Black Rat Snake	*Pantherophis obsoletus*	Jackson	No

Species	Scientific Name	County	*Oo* Detected
Black Rat Snake	*Pantherophis obsoletus*	Jackson	Yes
De Kay's snake	*Storeria dekayi*	Jackson	No
De Kay's snake	*Storeria dekayi*	Jackson	No
Common Garter Snake	*Thamnophis sirtalis*	Jackson	No
Common Garter Snake	*Thamnophis sirtalis*	Jackson	Yes
Copperhead	*Agkistrodon contortrix*	Marion	No
Worm Snake	*Carphophis amoenus*	Marion	No
Ring-necked Snake	*Diadophis punctatus*	Marion	No
Midland Water Snake	*Nerodia sipedon pleuralis*	Marion	No
Common Garter Snake	*Thamnophis sirtalis*	Marion	No
Worm Snake	*Carphophis amoenus*	Morgan	No
Copperhead	*Agkistrodon contortrix*	Overton	No
Worm Snake	*Carphophis amoenus*	Overton	No
Ring-necked Snake	*Diadophis punctatus*	Overton	No
Ring-necked Snake	*Diadophis punctatus*	Overton	No
Ring-necked Snake	*Diadophis punctatus*	Overton	No
Ring-necked Snake	*Diadophis punctatus*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	No
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
Northern Water Snake	*Nerodia sipedon*	Overton	Yes
De Kay's snake	*Storeria dekayi*	Overton	Yes
Ring-necked Snake	*Diadophis punctatus*	Putnam	No
Northern Water Snake	*Nerodia sipedon*	Putnam	Yes
Black Rat Snake	*Pantherophis obsoletus*	Putnam	Yes
De Kay's snake	*Storeria dekayi*	Putnam	No
De Kay's snake	*Storeria dekayi*	Putnam	Yes
Common Garter Snake	*Thamnophis sirtalis*	Putnam	No

Species	Scientific Name	County	*Oo* Detected
Common Garter Snake	*Thamnophis sirtalis*	Putnam	No
Common Garter Snake	*Thamnophis sirtalis*	Putnam	No
Copperhead	*Agkistrodon contortrix*	Rutherford	No
Black Racer	*Coluber constrictor*	Rutherford	Yes
Timber Rattlesnake	*Crotalus horridus*	Rutherford	No
Timber Rattlesnake	*Crotalus horridus*	Rutherford	Yes
Timber Rattlesnake	*Crotalus horridus*	Rutherford	Yes
Common Kingsnake	*Lampropeltis getula*	Rutherford	Yes
Common Kingsnake	*Lampropeltis getula*	Rutherford	Yes
Queen Snake	*Regina septemvittata*	Rutherford	Yes
Copperhead	*Agkistrodon contortrix*	Sevier	No
Copperhead	*Agkistrodon contortrix*	Sevier	No
Copperhead	*Agkistrodon contortrix*	Sevier	No
Timber Rattlesnake	*Crotalus horridus*	Sevier	No
Ring-necked Snake	*Diadophis punctatus*	Sevier	No
Ring-necked Snake	*Diadophis punctatus*	Sevier	No
Ring-necked Snake	*Diadophis punctatus*	Sevier	No
Ring-necked Snake	*Diadophis punctatus*	Sevier	No
Northern Water Snake	*Nerodia sipedon*	Sevier	No
Redbelly Snake	*Storeria occipitomaculata*	Sevier	No
Common Garter Snake	*Thamnophis sirtalis*	Sevier	No
Common Garter Snake	*Thamnophis sirtalis*	Sevier	No
Common Garter Snake	*Thamnophis sirtalis*	Sevier	No
Common Garter Snake	*Thamnophis sirtalis*	Sevier	No
Corn Snake	*Pantherophis guttatus*	Union	No
Copperhead	*Agkistrodon contortrix*	White	No
Worm Snake	*Carphophis amoenus*	White	No
Worm Snake	*Carphophis amoenus*	White	No
Worm Snake	*Carphophis amoenus*	White	No
Timber Rattlesnake	*Crotalus horridus*	White	No
Northern Water Snake	*Nerodia sipedon*	White	Yes
Common Garter Snake	*Thamnophis sirtalis*	White	Yes

Lab reports for additional collected samples are still pending.

Figure 9. Counties in which collections have been made for *O. ophiodiicola* testing.

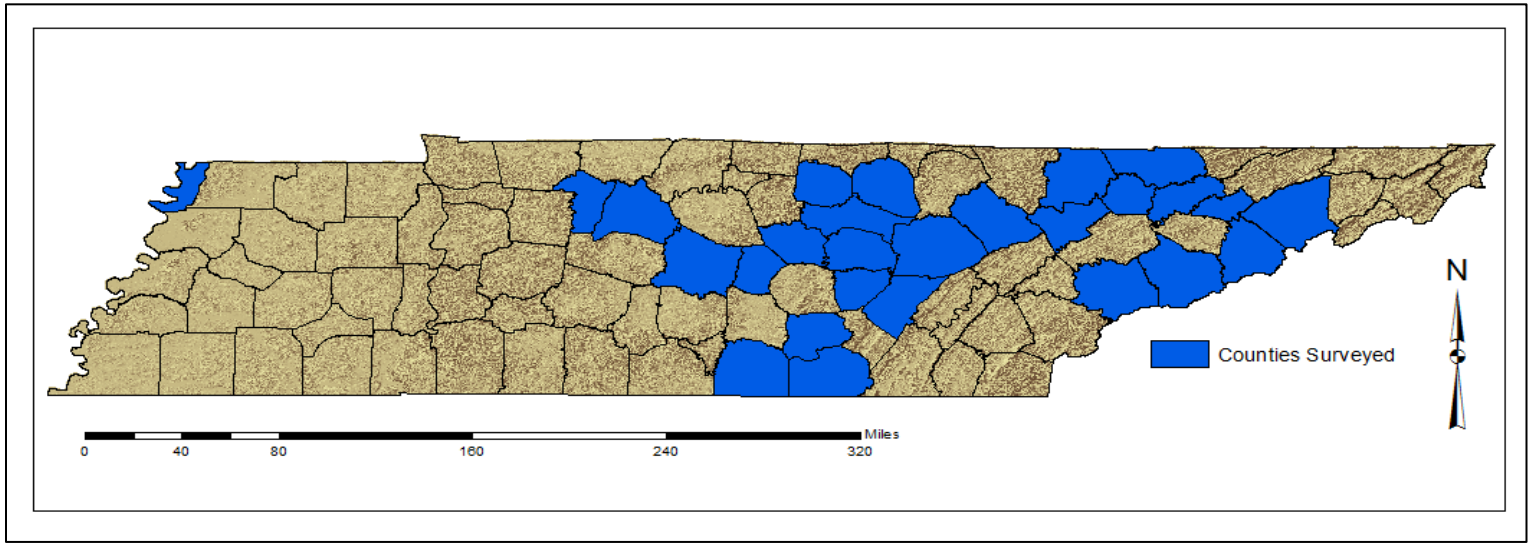
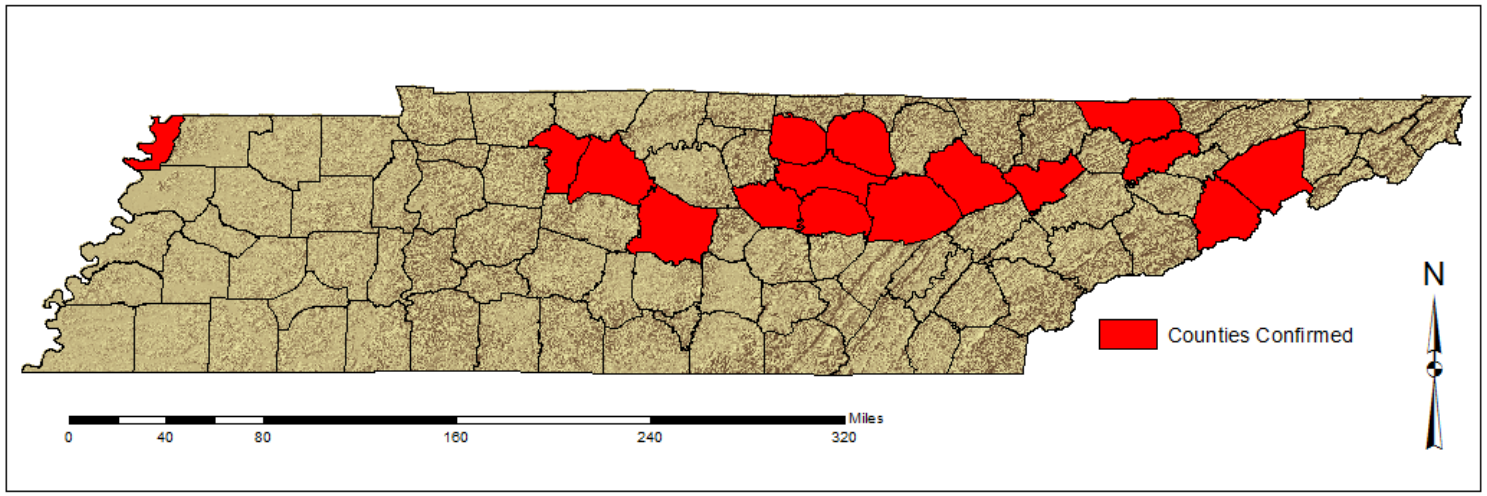


Figure 10. Counties in which *O. ophiodiicola* has been documented on snakes or SFD has been confirmed since 2012.



Reporting Potential SFD Observations

Observations of SFD can be made across the state throughout the year. Because of the geographic scope of any potential occurrence, collecting the samples necessary for testing can be problematic. However, maintaining a database of observations being made can be used to direct where sampling and monitoring can be targeted. The public is encouraged to submit records of any observations made to aid the efforts surrounding SFD. Any record submitted should contain the following information:

1. The date the observation is made
2. The exact location of the observation. GPS coordinates (dd.ddddd) must accompany any locality information submitted.
3. The species of snake observed.
4. The symptoms observed, to include unusual behavior.
5. Photographs of both the snake and symptoms, including any lesions, bumps or scabs observed.

Reports with the information above should be submitted to personnel in one of the following offices:

Statewide Office, Nashville
Roger Applegate
Wildlife Population Biologist
615-781-6616
Roger.Applegate@tn.gov

Region I Office, Jackson
Rob Colvin
Wildlife Biologist
731-423-5725
Rob.Colvin@tn.gov

Region II Office, Nashville
Josh Campbell
Wildlife Diversity Coordinator
615-781-6626
Josh.Campbell@tn.gov

Region III, Crossville
Chris Simpson
Wildlife Diversity Coordinator
931-484-9571
Chris.Simpson@tn.gov

Region IV, Morristown
Scott Dykes
Wildlife Diversity Coordinator
1-800-332-0900, ext. 112
Scott.Dykes@tn.gov

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Appendix A

- 2012 Diagnostic Services Reports

NATIONAL WILDLIFE HEALTH CENTER

6006 Schroeder Road
Madison, Wisconsin 53711-6223
608-270-2400 (FAX 608-270-2415)

DIAGNOSTIC SERVICES CASE UPDATE

CASE: 24216 **EPIZOO:** 12/18/2012

☐ Legal **INV NUM:**

FINDINGS TO DATE

Submitter:
Danny Bryan
Cumberland University
One Cumberland Square
Lebanon, TN 37087

Date Submitted: 11/6/2012

Specimen description/identification/Location:

ACC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER'S ID	COUNTY	STATE
001	Snake, Timber Rattlesnake	CARCASS			De Kalb	TN

Summary of Physical Characteristics

Event History

A single Timber rattlesnake was found dead near Saddle Dam at Center Hill Lake and collected on 10/5/12. It had skin lesions and swellings on the head especially around the left eye, loreal pit, and ventral body surface. This is the third specimen of the same species to be collected with similar clinical signs in the area since 2005; previous specimens died shortly after capture.

Snake was submitted to Matt Allendar (Univ. of IL) by Danny Bryan (Cumberland State University). Dr. Allendar forwarded the carcass to the NWHC for diagnostic evaluation.

Comment:

12/18/12: A single Timber rattlesnake carcass and skin shed were received chilled by NWHC on 11/6/12. External examination of the carcass revealed obvious signs of trauma, especially on the head. The advanced state of decomposition of the carcass including sloughing skin and maggot infestation was judged unsuitable for a full diagnostic necropsy. No grossly visible lesions consistent with fungal infection were observed on the skin and head although the poor condition of the carcass may have obscured these findings. Based on information provided from Dr. Allendar regarding possible antemortem facial lesions, the following samples were taken from the carcass for fungal testing: 1) facial skin, 2) spectacle, 3) pit skin, 4) chin skin.

In addition, the shed skin contained several thickened areas that are consistent with former skin lesions. Samples taken for fungal culture from the shed skin: 1) mid-body lateral scales #1, 2) mid-body lateral scales #2, 3) mid-body ventral scutes #1, 2) mid-body ventral scutes #2.

Fungal culture results are as follows:

A) Carcass
1) facial skin: yeast present (likely *Candida* sp.)
2) spectacle: *Candida* sp. (identified by DNA sequencing)
3) pit skin: yeast present (likely *Candida* sp.); *Galactomyces geotrichum* (identified by DNA sequencing)
4) chin skin:yeast present (likely *Candida* sp.)

B) Shed Skin
1) mid-body lateral scales #1: *Paecilomyces* sp. (identified by DNA sequencing)
2) mid-body lateral scales #2: no growth
3) mid-body ventral scutes #1: *Paecilomyces* sp. (identified by DNA sequencing)
4) mid-body ventral scutes #2: *Chrysosporium* sp. (identified by DNA sequencing); bacterial growth on fungal medium

Culture results should be interpreted with caution due to the carcass condition and potential for post-mortem growth. The *Candida* sp. and *Galactomyces geotrichum* isolated from the carcass likely represent fungi that colonized the animal post-mortem; they are not suspected pathogens. Some species of *Paecilomyces* are commonly isolated from the skin of healthy snakes and probably represent part of the normal skin flora. The *Chrysosporium* sp. isolated from the shed most closely matches *Chrysosporium ophiodiicola*, a suspected pathogen of wild snakes in the eastern U.S.

Appropriate cleaning and disinfection of equipment with a 10% bleach solution (1 part bleach, 9 parts water) between handling individual snakes in this area is advised to reduce the risk of cross-contamination of this suspected fungal pathogen in the population. AEB

Copies to:
Matt Allendar, Univ. of IL
Josh Campbell, TWRA

PRINTED December 18, 2012

FINDINGS TO DATE

If you have questions regarding this case, contact:

Anne E. Ballmann

Anne E. Ballmann , DVM, Ph.D.
Wildlife Disease Specialist
Phone: 608-270-2445 **E-Mail:** aballmann@usgs.gov

Diagnostic findings may not be used for publication without the pathologist's knowledge and consent.

Appendix B

- 2013 Diagnostic Services Reports

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-113
DATE RECEIVED April 18, 2013
DATE OF REPORT May 28, 2013

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snakes* (18) **SEX** N/A **AGE** * **WEIGHT** n/a
*Varies, see chart on page 2 for individual specimen details.

CASE HISTORY: Swabs, scape clips, and skin clips of various snake species were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in March and April 2013, from live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Vincent Cobb of Middle Tennessee State University. The snakes appeared generally healthy during sampling although a few had isolated skin lesions.
The samples were received on April 18, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: No *Chrysosporium ophiodiicola* detected.

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Chrysosporium ophiodiicola*. Although all affected snakes have so far been found to be infected with *C. ophiodiicola,* other fungi have also been isolated from these snakes and a definitive cause of SFD has yet to be identified. The snakes sampled during this time appeared healthy, so they were likely not harboring the fungus. However, as both SFD and *C. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out.
The results were reported to Mr. Applegate and Dr. Cobb by electronic communication on May 28, 2013.

WILDLIFE IMPLICATIONS: SFD is a fungal dermatitis causing deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are most often reported, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has greatly increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species. Other *Chrysosporium* species have been known to infect immunocompromised humans as well as captive snakes. Although *C. ophiodiicola* is implicated in SFD, co-infections with other pathogens are possible and a definitive link has not been established. Furthermore, it is not known at this time if C. ophiodiicola acts as a primary pathogen or if it causes opportunistic infections in susceptible individuals. Further research is needed to determine the etiology, pathogenesis, and population impact of SFD on various snake species.

PUBLIC HEALTH IMPLICATIONS: *C. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *C. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-113

Sample ID	Species	Date Collected	Type of sample	Body location	Age class
Ch 6a	Crotalus horridus	3/21/2013	surface swab	head, left lateral	subadult
Ch 6b	Crotalus horridus	3/21/2013	surface swab	head, right lateral	subadult
Ch 6c	Crotalus horridus	3/21/2013	scale clip	anterior ventral scale	subadult
Ch 31a	Crotalus horridus	3/30/2013	surface swab	head, left & right lateral	neonate
Ch 31b	Crotalus horridus	3/30/2013	scale clip	anterior ventral scale	neonate
Ch 13a	Crotalus horridus	4/9/2013	surface swab	head, left lateral	subadult
Ch 13b	Crotalus horridus	4/9/2013	surface swab	head, right lateral	subadult
Ch 5a	Crotalus horridus	4/10/2013	surface swab	head, left lateral	adult
Ch 5b	Crotalus horridus	4/10/2013	surface swab	head, right lateral	adult
Ch 5c	Crotalus horridus	4/10/2013	scale	head, preocular	adult
Ch 5d	Crotalus horridus	4/10/2013	cutaneous clip	body, left skin tag	adult
Ch 5e	Crotalus horridus	4/10/2013	cutaneous clip	body, right skin tag	adult
Ch5f	Crotalus horridus	4/10/2013	surface swab	clipped area of Ch 5e	adult
Ch 11a	Crotalus horridus	4/11/2013	surface swab	head, left lateral	adult
Ch 11b	Crotalus horridus	4/11/2013	surface swab	head, right lateral	adult
Po 1a	Pantherophis spiloides	3/16/2013	surface swab	head, left lateral	adult
Po 1b	Pantherophis spiloides	3/16/2013	surface swab	head, right lateral	adult
Cc 1a	Coluber constrictor	3/21/2013	surface swab	head, left & right lateral	adult
Cc 2a	Coluber constrictor	4/3/2013	surface swab	head, left & right lateral	subadult
Cc 3a	Coluber constrictor	4/5/2013	surface swab	head, left & right lateral	adult
Cc 4a	Coluber constrictor	4/5/2013	surface swab	head, left & right lateral	adult
Ac 1a	Agkistrodon contortix	4/13/2013	surface swab	head, left lateral	adult
Ac 2a	Agkistrodon contortix	4/13/2013	surface swab	head, right lateral	adult

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month. No sample had gross or cytologic appearance consistent with *C. ophiodiicola*. Four samples, Ch 5a, Ch5e, Po 1b, and Ac 1a had colonies that looked most like *C. ophiodiicola*. These samples were analyzed by polymerase chain reaction for a DNA match with *C. ophiodiicola*. The fungus was not detected in any sample.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-161
DATE RECEIVED May 16, 2013
DATE OF REPORT July 9, 2013

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snakes* (19) **SEX** n/a **AGE** * **WEIGHT** n/a

*Varies, see chart on page 3 for individual specimen details.

CASE HISTORY: Swabs, scale clips, and skin clips of various snake species were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in April and May 2013 from live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Vincent Cobb of Middle Tennessee State University. The snakes appeared generally healthy during sampling, although a few had isolated skin lesions.

The samples were received on May 16, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* detected in snakes Ch1, Rs1, and Rs2.
Ophidiomyces ophiodiicola was not detected from any other snakes.

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes so far have been found to be infected with *O. ophiodiicola,* other fungi have been isolated from these snakes, and a definitive cause of SFD has yet to be identified. Three snakes in this submission, one timber rattlesnake and two queen snakes, had positive cultures for *O. ophiodiicola*. Timber rattlesnakes are a frequently reported species among those diagnosed with SFD, and this individual had classic facial disfiguration lesions, according to the submitter. Previously, SFD had not been reported in queen snakes. The reported lesions on these two queen snakes were not similar to those typically described for SFD. This may be due to individual variation or a different manifestation of the disease in queen snakes. Progression of the disease is variable, and a few captive individuals have responded to antimicrobials and supportive care. As these snakes are being monitored, future samples will provide valuable insight into this emerging disease.

O. ophiodiicola was not found in any other individuals sampled. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out.

The initial results were reported to Mr. Applegate and Dr. Cobb by electronic communication on June 14, 2013, with updates on molecular results provided on June 20, June 28, and July 9, 2013.

WILDLIFE IMPLICATIONS: Snake Fungal Disease is a fungal dermatitis with deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased greatly since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species. Although *O. ophiodiicola* is implicated in SFD, co-infections with other pathogens are possible and a definitive, causative link has not been established: it is not known at this time if *O. ophiodiicola* acts as a primary pathogen, or if it causes opportunistic infections in susceptible individuals. Further research is needed to determine the etiology, pathogenesis, and population impact of SFD on various snake species.

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month. Five samples, Ch 1e, Ch 8b, Ps 1b, Rs 1b, and Rs 2b had colonies most consistent with *O. ophiodiicola* by morphoplogy and/or cytology. These samples were analyzed by polymerase chain reaction (PCR) for a DNA match with *O. ophiodiicola*. Positive results were found in Ch 1e, Rs 1b, and Rs 2b. These samples were then submitted for genomic sequencing and matched published genomic sequences of *O. ophiodiicola*.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 3

PAGE 3 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-161

Sample ID	Species	Date Collected	Type of sample	Body location	Age class
Ch 1a	Crotalus horridus	4/18/2013	surface swab	head, left lateral	adult
Ch 1b	Crotalus horridus	4/18/2013	surface swab	head, right lateral	adult
Ch 1c	Crotalus horridus	4/18/2013	surface swab	head, rostrum	adult
Ch 1d	Crotalus horridus	4/18/2013	surface swab	head, chin	adult
Ch 1e	Crotalus horridus	4/18/2013	scale clip	head, under chin	adult
Ch 1f	Crotalus horridus	4/18/2013	scale clip	left skin tag, 2cm from head	adult
Ch 3a	Crotalus horridus	4/24/2013	surface swab	head, left lateral	adult
Ch 3b	Crotalus horridus	4/24/2013	surface swab	head, right lateral	adult
Ch 3c	Crotalus horridus	4/24/2013	scale clip	head, under chin	adult
Ch 3d	Crotalus horridus	4/24/2013	cutaneous clip	head, under chin	adult
Ch 5	Crotalus horridus	4/10/2013	surface swab	head	adult
Ch 8a	Crotalus horridus	4/18/2013	surface swab	head, left lateral	adult
Ch 8b	Crotalus horridus	4/18/2013	surface swab	head, right lateral	adult
Ch 10a	Crotalus horridus	4/17/2013	surface swab	head, left lateral	adult
Ch 10b	Crotalus horridus	4/17/2013	surface swab	head, right lateral	adult
Ch 10c	Crotalus horridus	4/17/2013	scale clip	48th ventral scale from head	adult
Ch 10d	Crotalus horridus	4/17/2013	scale clip	53rd ventral scale from head	adult
Ch 14-1a	Crotalus horridus	4/18/2013	surface swab	head, left lateral	adult
Ch 14-1b	Crotalus horridus	4/18/2013	surface swab	head, right lateral	adult
Ch 14-2a	Crotalus horridus	4/19/2013	surface swab	head, left lateral	subadult
Ch 14-2b	Crotalus horridus	4/19/2013	surface swab	head, right lateral	subadult
Ch 15a	Crotalus horridus	4/22/2013	surface swab	head, left lateral	subadult
Ch 16a	Crotalus horridus	4/23/2013	surface swab	head, left lateral	subadult
Ch 16b	Crotalus horridus	4/23/2013	surface swab	head, right lateral	subadult
Ch 17a	Crotalus horridus	4/25/2013	surface swab	head, left lateral	adult
Ch 17b	Crotalus horridus	4/25/2013	surface swab	head, right lateral	adult
Ch 18a	Crotalus horridus	5/4/2013	surface swab	head, left lateral	adult
Ch 18b	Crotalus horridus	5/4/2013	surface swab	head, right lateral	adult
Ch 18c	Crotalus horridus	5/4/2013	scale clip	scale	adult
Cs 5a	Coluber constrictor	4/30/2013	surface swab	head	adult
Ps 2a	Pantherophis spiloides	4/17/2013	surface swab	head, left lateral	adult
Ps 2b	Pantherophis spiloides	4/17/2013	surface swab	head, right lateral	adult
Lt 1a	Lampropeltis triangulum	5/2/2013	surface swab	head, left lateral	adult
Lt 1b	Lampropeltis triangulum	5/2/2013	surface swab	head, right lateral	adult
Ns 2a	Nerodia sipedon	5/4/2013	surface swab	head, nose	adult
Lg 1a	Lampropeltis getula	5/11/2013	surface swab	head, left lateral	subadult
Lg 1b	Lampropeltis getula	5/11/2013	surface swab	head, right lateral	subadult
Oa 1a	Opheodrys aestivus	5/13/2013	surface swab	head, left lateral	adult
Oa 1b	Opheodrys aestivus	5/13/2013	surface swab	head, right lateral	adult
Rs 1a	Regina septemvitatta	5/14/2013	surface swab	head, both sides	adult
Rs 1b	Regina septemvitatta	5/14/2013	surface swab	under loose scales, midbody	adult
Rs 1c	Regina septemvitatta	5/14/2013	surface swab	scales from chin	adult
Rs 2a	Regina septemvitatta	5/14/2013	surface swab	scales from chin	adult
Rs 2b	Regina septemvitatta	5/14/2013	surface swab	rough scales on base of tail	adult

Highlighted samples represent those found positive for *O. ophiodiicola*.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-260
DATE RECEIVED August 14, 2013
DATE OF REPORT October 23, 2013

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snakes* (12) **SEX** n/a **AGE** * **WEIGHT** n/a
*Varies, see chart on page 2 for individual specimen details.

CASE HISTORY: Swabs of various snake species were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in May to August 2013 from live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Vincent Cobb of Middle Tennessee State University. The snakes appeared generally healthy during sampling, although a few had isolated skin lesions.

The samples were received on August 14, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* not detected.

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes have so far been found to be infected with *O. ophiodiicola,* other fungi have also been isolated from these snakes and a definitive cause of SFD has yet to be identified.

O. ophiodiicola was not found in any of the current submissions. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out. Additionally, infection occurs in the dermis and spores may not be detected using superficial swabs. As these snakes are being monitored, future samples will provide valuable insight into this newly emerging disease.

The initial results were reported to Mr. Applegate and Dr. Cobb by electronic communication on August 14, 2013, with regular updates provided on culture and molecular results.

WILDLIFE IMPLICATIONS: SFD is a fungal dermatitis causing deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are most often reported, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has greatly increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species. Although *O. ophiodiicola* is implicated in SFD, co-infections with other pathogens are possible and a definitive link has not been established. Futhermore, it is not known at this time if *O. ophiodiicola* acts as a primary pathogen or if it causes opportunistic infections in susceptible individuals. Further research is needed to determine the etiology, pathogenesis, and population impact of SFD on various snake species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-260

Sample ID	Species	Date Collected	Type of sample	Body location	Age class
Ch1g	Crotalus horridus	8/2/2013	surface swab	head, left lateral	adult
Ch1h	Crotalus horridus	8/2/2013	surface swab	head, right lateral	adult
Ch1i	Crotalus horridus	8/2/2013	surface swab	head, rostrum	adult
Ch4a	Crotalus horridus	5/16/2013	surface swab	head, left lateral	adult
Ch4b	Crotalus horridus	5/16/2013	surface swab	head, right lateral	adult
Ch4c	Crotalus horridus	8/2/2013	surface swab	head, left lateral	adult
Ch4d	Crotalus horridus	8/2/2013	surface swab	head, right lateral	adult
Ch5g	Crotalus horridus	8/2/2013	surface swab	head, left lateral	adult
Ch5h	Crotalus horridus	8/2/2013	surface swab	head, right lateral	adult
Ch5i	Crotalus horridus	8/2/2013	surface swab	head, rostrum	adult
Ch6d	Crotalus horridus	8/5/2013	surface swab	head, left lateral	adult
Ch6e	Crotalus horridus	8/5/2013	surface swab	head, right lateral	adult
Ch10e	Crotalus horridus	8/2/2013	surface swab	head, left lateral	adult
Ch10f	Crotalus horridus	8/2/2013	surface swab	head, right lateral	adult
Ch10g	Crotalus horridus	8/2/2013	surface swab	head, rostrum	adult
Ch14e	Crotalus horridus	8/2/2013	surface swab	head, left lateral	subadult
Ch14f	Crotalus horridus	8/2/2013	surface swab	head, right lateral	subadult
Ch15b	Crotalus horridus	8/5/2013	surface swab	head, left lateral	subadult
Ch15c	Crotalus horridus	8/5/2013	surface swab	head, right lateral	subadult
Ch16c	Crotalus horridus	8/5/2013	surface swab	head, left lateral	subadult
Ch 19a	Crotalus horridus	6/8/2013	surface swab	head, left lateral	adult
Ch19b	Crotalus horridus	6/8/2013	surface swab	head, right lateral	adult
Ch19c	Crotalus horridus	8/5/2013	surface swab	head, left lateral	adult
Ch19d	Crotalus horridus	8/5/2013	surface swab	head, right lateral	adult
CC6a	Coluber constrictor	5/22/2013	surface swab	head, left lateral	adult
Cc6b	Coluber constrictor	5/22/2013	surface swab	head, right lateral	adult
Lg2a	Lampropeltis triangulum	5/15/2013	surface swab	head, left lateral	adult
Lg2b	Lampropeltis triangulum	5/15/2013	surface swab	head, right lateral	adult
Lg3a	Lampropeltis triangulum	5/28/2013	surface swab	head, left lateral	adult
Lg3b	Lampropeltis triangulum	5/28/2013	surface swab	head, right lateral	adult

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month. Eight samples, Ch1i, Ch4d, Ch5g, Ch6d, Ch10f, Ch14e, Ch15b, and Ch16c had colonies suspicious for *O. ophiodiicola* by morphoplogy and/or cytology. These samples were analyzed by polymerase chain reaction (PCR) for a DNA match with *O. ophiodiicola*. All swabs were also assayed. No O. ophiodiicola DNA was detected by PCR from the cultures or swabs.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-325
DATE RECEIVED September 13, 2013
DATE OF REPORT November 5, 2013

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snakes* (7) **SEX** n/a **AGE** * **WEIGHT** n/a
*Varies, see chart on page 3 for individual specimen details.

CASE HISTORY: Skin swabs from various snake species were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in August 2013 from live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Vincent Cobb of Middle Tennessee State University. The snakes appeared generally healthy during sampling. No skin lesions were visible.

The samples were received on September 13, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* not detected

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes have so far been found to be infected with *O. ophiodiicola,* other fungi have also been isolated from these snakes and a definitive cause of SFD has yet to be identified.

O. ophiodiicola was not found in any of the current submissions. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out. Additionally, infection occurs in the dermis and spores may not be detected using superficial swabs. As these snakes are being monitored, future samples will provide valuable insight into this newly emerging disease.

The initial results were reported to Mr. Applegate and Dr. Cobb by electronic communication on August 14, 2013, with regular updates provided on culture and molecular results.

WILDLIFE IMPLICATIONS: SFD is a fungal dermatitis causing deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are most often reported, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has greatly increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 3

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-325

Sample ID	Species	Date Collected	Type of sample	Body location	Age class
Ch8c	*Crotalus horridus*	8/17/2013	surface swab	head, left lateral	adult
Ch8d	*Crotalus horridus*	8/17/2013	surface swab	head, right lateral	adult
Ch11c	*Crotalus horridus*	8/17/2013	surface swab	head, right lateral	adult
Ch11d	*Crotalus horridus*	8/17/2013	surface swab	head, left lateral	adult
Ch17c	*Crotalus horridus*	8/14/2013	surface swab	head, left lateral	adult
Ch18d	*Crotalus horridus*	8/12/2013	surface swab	head, left lateral	adult
Jj1a	*Crotalus horridus*	8/30/2013	surface swab	head, left lateral	adult
Jj1b	*Crotalus horridus*	8/30/2013	surface swab	head, right lateral	adult
Jj2a	*Crotalus horridus*	8/30/2013	surface swab	head, left lateral	adult
Jj2b	*Crotalus horridus*	8/30/2013	surface swab	head, right lateral	adult
Lt2a	*Lampropeltis triangulum*	8/17/2013	surface swab	head	adult

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month. Three samples, Ch11c, Jj1a, and Jj2a had colonies suspicious for *O. ophiodiicola* by morphoplogy and/or cytology. These samples were analyzed by polymerase chain reaction (PCR) for a DNA match with *O. ophiodiicola*. All swabs were also assayed. No O. ophiodiicola DNA was detected by PCR from the cultures or swabs.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-462
DATE RECEIVED November 8, 2013
DATE OF REPORT December 16, 2013

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snake* (21) **SEX** n/a **AGE** * **WEIGHT** n/a
*Varies, see chart on page 3 for individual specimen details.

CASE HISTORY: Skin swabs from various snake species were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in August 2013 from live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Vincent Cobb of Middle Tennessee State University. The snakes appeared generally healthy during sampling. Mild skin lesions were visible on a few snakes. The individuals with visible lesions that had been previously observed were less severely affected than when those individual snakes had been sampled earlier in the year.

The samples were received on November 8, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* not detected

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes have so far been found to be infected with *O. ophiodiicola,* other fungi have also been isolated from these snakes and a definitive cause of SFD has yet to be identified.

O. ophiodiicola was not found in any of the current submissions. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out. Additionally, infection occurs in the dermis and spores may not be detected using superficial swabs. As these snakes are being monitored, future samples will provide valuable insight into this newly emerging disease.

Receipt of samples was reported to Mr. Applegate and Dr. Cobb by electronic communication on November 8, 2013, with regular updates provided on culture and molecular results.

WILDLIFE IMPLICATIONS: SFD is a fungal dermatitis causing deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are most often reported, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has greatly increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-462

Sample ID	Species	Date Collected	Type of Sample	Location	Age Class	Notes
CH1j	*Crotalus horridus*	8-Oct	surface swab	head, left lateral	adult	facial evidence, milder than CH5
CH1k	*Crotalus horridus*	8-Oct	surface swab	head, left nostril	adult	facial evidence, milder than CH5
CH1l	*Crotalus horridus*	8-Oct	surface swab	head, right lateral	adult	facial evidence, milder than CH5
CH4e	*Crotalus horridus*	8-Oct	surface swab	head, left lateral	adult	no visible problems
CH4f	*Crotalus horridus*	8-Oct	surface swab	head, right lateral	adult	no visible problems
CH5j	*Crotalus horridus*	7-Oct	surface swab	head, left lateral	adult	facial evidence, better than midsummer
CH5k	*Crotalus horridus*	7-Oct	surface swab	head, right lateral	adult	facial evidence, better than midsummer
CH5l	*Crotalus horridus*	7-Oct	surface swab	under facial scale	adult	white globules under facial scale
CH6g	*Crotalus horridus*	29-Oct	surface swab	head, left lateral	subadult	no visible problems
CH6f	*Crotalus horridus*	29-Oct	surface swab	head, right lateral	subadult	no visible problems
CH7a	*Crotalus horridus*	28-Oct	surface swab	head, left lateral	adult	no visible problems
CH7b	*Crotalus horridus*	28-Oct	surface swab	head, right lateral	adult	no visible problems
CH8e	*Crotalus horridus*	9-Oct	surface swab	head, left lateral	adult	no visible problems
CH8f	*Crotalus horridus*	9-Oct	surface swab	head, right lateral	adult	no visible problems
CH8g	*Crotalus horridus*	9-Oct	scale clip	facial scale	adult	no visible problems
CH10h	*Crotalus horridus*	6-Oct	surface swab	head, left lateral	adult	no visible problems
CH10i	*Crotalus horridus*	6-Oct	surface swab	head, right lateral	adult	no visible problems
CH11e	*Crotalus horridus*	5-Oct	surface swab	head, left lateral	adult	no visible problems
CH11f	*Crotalus horridus*	5-Oct	surface swab	head, right lateral	adult	no visible problems
CH14g	*Crotalus horridus*	30-Oct	surface swab	head, left lateral	subadult	none on head
CH14h	*Crotalus horridus*	30-Oct	surface swab	head, right lateral	subadult	none on head
CH14i	*Crotalus horridus*	30-Oct	scale clip	ant. ventral body	subadult	normal scale clip under necrotic scale
CH14j	*Crotalus horridus*	30-Oct	scale clip	ant. ventral body	subadult	small brown necrotic external scale
CH14k	*Crotalus horridus*	30-Oct	scale clip	ant. ventral body	subadult	small brown necrotic external scale
CH15d	*Crotalus horridus*	5-Oct	surface swab	head, left lateral	subadult	no visible problems
CH15e	*Crotalus horridus*	5-Oct	surface swab	head, right lateral	subadult	no visible problems
CH16d	*Crotalus horridus*	25-Oct	surface swab	head, left lateral	subadult	no visible problems
CH16e	*Crotalus horridus*	25-Oct	surface swab	head, right lateral	subadult	no visible problems
CH17d	*Crotalus horridus*	14-Aug	surface swab	head, right lateral	adult	no visible problems
CH17e	*Crotalus horridus*	7-Oct	surface swab	head, left lateral	adult	no visible problems
CH17f	*Crotalus horridus*	7-Oct	surface swab	head, right lateral	adult	no visible problems
CH18e	*Crotalus horridus*	12-Aug	surface swab	head, right lateral	adult	no visible problems
CH18f	*Crotalus horridus*	4-Oct	surface swab	head, left lateral	adult	no visible problems
CH18g	*Crotalus horridus*	4-Oct	surface swab	head, right lateral	adult	no visible problems
CH19e	*Crotalus horridus*	4-Oct	surface swab	head, left lateral	adult	no visible problems
CH19f	*Crotalus horridus*	4-Oct	surface swab	head, right lateral	adult	no visible problems
CH608a	*Crotalus horridus*	27-Oct	surface swab	head, left lateral	adult	no visible problems
CH608b	*Crotalus horridus*	27-Oct	surface swab	head, right lateral	adult	no visible problems
CH534a	*Crotalus horridus*	1-Nov	surface swab	head, both sides	subadult	no visible problems
CH808a	*Crotalus horridus*	1-Nov	surface swab	head, both sides	juvenile	no visible problems
CH881a	*Crotalus horridus*	1-Nov	surface swab	head, both sides	juvenile	no visible problems
CC7	*Coluber constrictor*	2-Oct	surface swab	head	adult	no visible problems
CC8	*Coluber constrictor*	11-Oct	surface swab	head	adult	no visible problems
AC2a	*Agkistrodon contortrix*	4-Nov	surface swab	head, rostrum	adult	no visible problems

PAGE 3 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC13-462

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month. Three samples, CH14f, CH15d, and CH808, had colonies suspicious for *O. ophiodiicola* by morphoplogy and/or cytology. These samples were analyzed by polymerase chain reaction (PCR) for a DNA match with *O. ophiodiicola*. All swabs were also assayed. No *O. ophiodiicola* DNA was detected by PCR from the cultures or swabs.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC13-483
DATE RECEIVED November 26, 2013
DATE OF REPORT December 13, 2013

STATE TN **COUNTY** Franklin **AREA** Williamson

SPECIES (NO.) Gray Ratsnake (1) **SEX** unk **AGE** Adult **WEIGHT** n/a
*Varies, see chart on page 3 for individual specimen details.

CASE HISTORY: Skin swabs from a gray ratsnake were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency for Snake Fungal Disease testing. The snake was observed with rough and puffy scales on the head and 3-4 small sores less than 1 cm in diameter on both sides of the body on November 20, 2013, by a private citizen who alerted Vincent Cobb of Middle Tennessee State University.

The samples were received on November 26, 2013, and immediately plated for fungal culture.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* not detected

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes have so far been found to be infected with *O. ophiodiicola,* other fungi have also been isolated from these snakes and a definitive cause of SFD has yet to be identified.

O. ophiodiicola was not found in the current submission. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out. Additionally, infection occurs in the dermis and spores may not be detected using superficial swabs. As these snakes are being monitored, future samples will provide valuable insight into this newly emerging disease.

Receipt of samples was reported to Mr. Applegate and Dr. Cobb by electronic communication on November 13, 2013, with regular updates provided on culture and molecular results.

WILDLIFE IMPLICATIONS: SFD is a fungal dermatitis causing deep infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are most often reported, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has greatly increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 70°F and is not thought to be infectious to humans.

LIVESTOCK IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes and other reptiles kept as pets or on display may potentially be at risk for infection.

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for several weeks. These samples were analyzed by polymerase chain reaction (PCR) for a DNA match with *O. ophiodiicola*. All swabs were also assayed by PCR. No *O. ophiodiicola* DNA was detected by PCR from the cultures or swab

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** John R. Fischer, DVM, PhD

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Appendix C

- 2014 Diagnostic Services Reports

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
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CASE NUMBER CC14-49
DATE RECEIVED January 31, 2014
DATE OF REPORT April 25, 2014

STATE TN **COUNTY** Rutherford **AREA** Flat Rock Cedar Glade & Barren Natural Area

SPECIES (NO.) Timber Rattlesnake* (3) **SEX** n/a **AGE** * **WEIGHT** n/a
*Varies, see chart on page 2 for individual specimen details.

CASE HISTORY: Tissues and skin swabs from three timber rattlesnakes were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in December 2013 and January 2014 from primarily live, free-ranging snakes for a Snake Fungal Disease surveillance effort by Dr. Vincent Cobb of Middle Tennessee State University. One snake (Ch 5) had facial swelling that was observed during previous monitoring efforts; it was found dead of suspected predation in December. The other two snakes were sampled due to observed skin swellings while they were seen basking in January 2014. The samples were received on January 31, 2014, and immediately processed.

FINAL DIAGNOSIS: Snake fungal disease, snake CH 10; *Ophidiomyces ophiodiicola* isolated
Fungal dermatitis of unknown species, snake CH 377
Trauma, snake CH 5

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes so far have been found to be infected with *O. ophiodiicola,* other fungi have been isolated from these snakes and a definitive cause of SFD has yet to be identified.

O. ophiodiicola was detected only in snake CH10. However, as both SFD and *O. ophiodiicola* are poorly characterized at this time, early infection cannot be ruled out in the other submitted snakes. As these snakes are being monitored, future samples will provide valuable insight into this newly emerging disease. Additional research is ongoing to further understand SFD and *O. ophiodiicola.* Additional results will be forwarded in an addendum if they are significant.

Receipt of samples was reported to Mr. Applegate and Dr. Cobb by electronic communication on February 10, 2014, with regular updates provided with final molecular results reported on April 11, 2014.

WILDLIFE IMPLICATIONS: SFD is a dermatitis with deep fungal infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Cobb, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC14-49
HISTO NUMBER CC14-49

Sample ID	Date Collected	Type of Sample	Location	Age class	Notes
CH5m	19-Dec	body x-section	midbody	adult	no SFD lesions on carcass
CH10j	23-Jan	surface swab	facial swab	adult	swab of swollen facial area
CH1l0k	23-Jan	tissue cut	face	adult	tissue from facial swelling
CH10l	23-Jan	subcut tissue	face	adult	white globular subcutaneous tissue
CH10m	23-Jan	tissue cut	neck	adult	tissue from neck swelling
CH10n	23-Jan	subcut tissue	neck	adult	white globular subcutaneous tissue
CH10o	23-Jan	brown scale	body	adult	sloughing body scale
CH10p	23-Jan	brown scale	body	adult	sloughing body scale
CH377a	13-Oct	surface swab	body swab	juvenile	swab of crusty browned body scales
CH377b	27-Jan	brown scale	body, spot 1	juvenile	sloughed body scales at local swelling
CH377c	27-Jan	tissue cut	body, spot 1	juvenile	tissue from under removed scale
CH377d	27-Jan	brown scale	body, spot 2	juvenile	sloughed body scales at local swelling
CH377e	27-Jan	tissue cut	body, spot 2	juvenile	tissue from under removed scale

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month.

Colonies suspicious for *Ophidiomyces ophiodiicola* were isolated from sample CH10k. The presence of *O. ophiodiicola* was detected by polymerase chain reaction (PCR) and confirmed by genomic sequencing.

O. ophiodiicola was not detected by culture or PCR from the other snakes.

MICROSCOPIC EXAMINATION:

CH5 (CC14-49A): Large numbers of mixed gram-negative and gram-positive bacterial colonies are present on the surface of the skin and throughout the dermis and underlying musculature. Fungi are not present on PAS staining. No associated inflammation is present.

CH10 (CC14-49B): The epidermis is mildly hypertrophied. Moderate numbers of heterophils and melanomacrophages are randomly distributed throughout the epidermis and dermis. Multifocal areas of dermis are grossly expanded by dense nodules of eosinophilic proteinaceous debris containing thin-walled, irregular, non-parallel, pauciseptate fungal hyphae approximately 1-2microns in diameter that stain with PAS staining. The hyphaie are surrounded by fibrous connective tissue infiltrated by small numbers of heterophils, macrophages, lymphocytes and plasma cells. Fungal hyphae occasionally infiltrate the epidermis. Large numbers of mixed gram-positive bacteria are present on the surface.

CH377 (CC14-49C): The epidermis is markedly hyperplastic and mildly hyperkeratotic. Frequent ballooning degeneration of the keratocytes is present. The epidermis is occasionally infiltrated by small numbers of thin-walled, irregular, non-parallel, pauciseptate fungal hyphae approximately 3-4microns in diameter. Subcutaneous tissue is markedly expanded with large amounts of poorly ordered connective tissue infiltrated by large numbers of lymphocytes, plasma cells, macrophages and occasional heterophils. Small numbers of gram negative bacteria are present on the surface. Large numbers of fungal hyphae are randomly distributed throughout the subcutaneous tissue. Multifocal areas of cellular debris are randomly distributed throughout the dermis. A focally extensive area of necrotic debris surrounded by fibrous connective tissue is present.

MORPHOLOGIC DIAGNOSES:

CH5: Postmortem bacterial overgrowth.

CH10: Granulomatous and heterophilic dermatitis, multifocal, severe, chronic, with intralesional fungal hyphae.

CH377: Dermal hyperplasia, multifocal, severe, chronic, with intralesional fungal hyphae and focal granuloma.

DIAGNOSTIC SERVICES SECTION

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
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FINAL REPORT

CASE NUMBER CC14-138
DATE RECEIVED April 25, 2014
DATE OF REPORT June 25, 2014

STATE TN **COUNTY** Greene **AREA** Lick Cree Bottoms Wildlife Management Area

SPECIES (NO.) Black Racer (1) **SEX** unk **AGE** unk **WEIGHT** n/a

CASE HISTORY: Biopsy samples from a live-captured, black racer were submitted by Chris Ogle of the Tennessee Wildlife Resources Agency for Snake Fungal Disease testing. The snake was found with facial swelling and discolored scales on April 23, 2014. It was released after the appropriate samples were obtained. The samples were received on February 25, 2014, and immediately processed.

FINAL DIAGNOSIS: Severe bacterial and fungal dermatitis

COMMENTS: Severe heterophilic dermatitis was present in this snake, but fungal and bacterial growth was restricted to the surface, and *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola* could not be isolated on fungal culture was not detected by PCR. Snake Fungal Disease (SFD) is generally characterized by a deep fungal infection with invasion of the dermis. SFD is an emerging disease associated with the newly described fungus *O. ophiodiicola*. Although all affected snakes so far have been found to be infected with *O. ophiodiicola,* other fungi have been isolated from these snakes, and a definitive cause of SFD has yet to be determined. As both SFD and *O. ophiodiicola* are poorly characterized at this time, and early infection cannot be completely ruled out, because *O. ophiodiicola* is a slow growing fungus and is challenging to isolate among many other natural microfauna present on snakes.

Receipt of samples was reported to Mr. Ogle by electronic mail on April 8, 2014, with regular updates provided until final molecular results were obtained on June 13, 2014.

WILDLIFE IMPLICATIONS: SFD is a dermatitis with deep fungal infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *O. ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *O. ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc

DISTRIBUTION: SCWDS File, Ogle, Ratajczak, Sumners, Applegate, Cobb, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 3

PAGE 2 – SCWDS -- LABORATORY RESULTS

FINAL REPORT

CASE NUMBER CC14-138
HISTO NUMBER W14-170

MICROSCOPIC EXAMINATION:

Skin: The surface of the submitted sample of ulcerated skin is covered in a thick serocellular crust containing numerous mixed bacterial colonies and large numbers of occasionally branching at acute angles, septate fungal hyphae approximately 3-4 micron in diameter with thin, irregular, non-parallel walls that stain with GMS and PAS and are admixed with heterophils, fibrin and necrotic debris. The epidermis is moderately hypertrophied and is infiltrated by large numbers of heterophils and free eosinophilic granules. Occasional keratinocytes have ballooning change (intracellular edema) as well as prominent intercellular connections (intercellular edema or spongiosis). Aggregates of heterophils, lymphocytes, plasma cells and macrophages are present throughout the dermis. The dermis and underlying subcutis is thickened by fibrous connective tissue.

MORPHOLOGIC DIAGNOSES:

Skin: Heterophilic, lymphoplasmacytic and granulomatous dermatitis multifocal, severe, chronic with intralesional fungal hyphae and mixed bacterial colonies.

MICROBIOLOGY: Samples from multiple areas of skin were plated for fungal culture and allowed to grow for at least one month.

Fungal growth could not be identified and was of uncertain significance. No colonies consistent with *Ophidiomyces ophiodiicola* (Oo) were isolated. Oo was not detected by polymerase chain reaction (PCR).

DIAGNOSTIC SERVICES SECTION

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FINAL REPORT

CASE NUMBER CC14-139
DATE RECEIVED April 28, 2014
DATE OF REPORT June 20, 2014

STATE TN **COUNTY** Rutherford **AREA** Various

SPECIES (NO.) Snakes* (3) **SEX** n/a **AGE** * **WEIGHT** n/a

TWRA ID	SCWDS ID	Date Collected	Type of Sample	Location	Age class	Notes
CH7-a2014	CC14-138A	16-Apr	tissue cut	head, chin	adult	swollen chin area, right
CH7-b2014	CC14-138A	17-Apr	tissue cut	head, chin	adult	swollen chin area, left
CC1-a2014	CC14-138B	17-Apr	tissue cut	head, chin	juvenile	one brown spot on chin
LT1-a2014	CC14-138C	17-Apr	tissue cut	posterior body	adult	rough, brown scales, left
LT1-b2014	CC14-138C	18-Apr	tissue cut	posterior body	adult	rough, brown scales, right
LT1-c2014	CC14-138C	19-Apr	tissue cut	posterior body	adult	rough, brown scales, right

CASE HISTORY: Biopsy samples and swabs of skin from three live-captured snakes were submitted by Roger Applegate of the Tennessee Wildlife Resources Agency. The samples were collected in April 2014 from live, free-ranging snakes as part of a Snake Fungal Disease surveillance program by Dr. Vincent Cobb of Middle Tennessee State University. One snake had a swollen chin and the others had rough, brown scales.

The samples were received on April 28, 2014 and immediately processed.

FINAL DIAGNOSIS: Snake C (LT1): Snake Fungal Disease (*Ophidiomyces ophiodiicola* confirmed).
Snakes A (CH7) and B (CC1): Fungal dermatitis (*Ophidiomyces ophiodiicola* suspected).

COMMENTS: Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*. Although all affected snakes so far have been found to be infected with *O. ophiodiicola,* other fungi have been isolated from these snakes and a definitive cause of SFD has yet to be determined.

Ophidiomyces ophiodiicola was detected only in snake LT 1 by PCR and culture. However fungal hyphae with identical microscopic morphology were noted in all three snakes submitted. Follow up information on these submissions would be appreciated to help us better understand this emerging disease. Additional research is ongoing to further understand SFD and *O. ophiodiicola*.

Receipt of samples was reported to Mr. Applegate and Dr. Cobb by electronic communication on April 29, 2014, with regular updates provided until final molecular results were reported on June 13, 2014.

WILDLIFE IMPLICATIONS: SFD is a dermatitis with deep fungal infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*),

massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *Ophidiomyces ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Lisa Last, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc

DISTRIBUTION: SCWDS File, Ratajczak, Sumners, Applegate, Cobb, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 3

FINAL REPORT

CASE NUMBER CC14-139
HISTO NUMBER W14-171 A-C

MICROSCOPIC EXAMINATION:

CH7 (CC14-139A): A focally extensive area of the epidermis is ulcerates and replaced by a thick serocellular crust composed of fibrin and cellular debris. Adjacent keratinocytes have large clear vacuoles within their cytoplasm. Large numbers of predominantly gram-negative coccobacilli are present on the surface of the skin with mixed bacterial colonies present within the superficial keratin and often deep within foci of necrosis. Fungal hyphae are present within the overlying keratin are approximately 2-4microns in diameter, septate and occasionally branch at acute angles with non-dichotomous branching and have irregular, non-parallel, thin walls. Fungal hyphae stain positive with PAS and GMS staining. The dermis is expanded by edema and focally extensive areas of lytic and coagulative necrosis surrounded by lymphocytes, macrophages and degenerate heterophils.

CC1 (CC14-139B): This sample is primarily composed of ulcerated skin replaced by necrotic debris admixed with heterophils, fibrin, and large numbers of thin-walled, irregular, non-parallel, septate fungal hyphae with rare acute angle, non-dichotomous branching that are approximately 2-4 microns in diameter that stain with PAS and GMS. A small portion of remaining dermis is infiltrated by large numbers of melanomacrophages and fibrous connective tissue. Large numbers of gram negative rods and large chain-forming gram-positive coccobaccili are present throughout the necrotic material.

CLT1 (CC14-179C): The epidermis is mildly hyperplastic surrounding an extensive focus of ulceration and is covered in a thick serocellular crust. Large numbers of thin-walled, irregular, non-parallel, acute angle, non-dichotomous branching, septate fungal hyphae approximately 2-4 microns in diameter that stain with PAS and GMS are present in the crust and rarely infiltrate the epidermis. Occasional degenerated heterophils are noted transmigrating through the epidermis. A large number of cells with intracytoplasmic brown pigment are present in the basal layer of the epidermis. Large numbers of mixed bacteria are present on the surface and within the serocellular crust. Multiple colonies of gram negative cocci surround the myofibers of the panniculus muscle with occasional colonies present within the dermis.

MORPHOLOGIC DIAGNOSES:

CH7 (CC14-139A): Necroulcerative dermatitis, focal, severe, chronic, with intralesional mixed bacterial colonies and fungal hyphae.

CC1 (CC14-139B): Necroulcerative dermatitis, diffuse, severe, chronic, with intralesional bacterial colonies and fungal hyphae.

CLT1 (CC14-179C): Necroulcerative dermatitis diffuse, severe, chronic, with intralesional bacterial colonies and fungal hyphae.

MICROBIOLOGY: All samples were plated for fungal culture at SCWDS and allowed to grow for at least one month.

Colonies suspicious for *Ophidiomyces ophiodiicola* were isolated from sample LT1-a2014. The presence of *O. ophiodiicola* was detected by polymerase chain reaction (PCR) and confirmed by genomic sequencing.

O. ophiodiicola was not detected by culture or PCR from the other snakes. Early growth of a colony isolated from CC1-a2014 was suggestive of *O. ophiodiicola,* but later growth and spore morphology were not consistent for definitive diagnosis.

DIAGNOSTIC SERVICES SECTION

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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FINAL REPORT

CASE NUMBER CC14-284
DATE RECEIVED September 25, 2014
DATE OF REPORT November 6, 2014

STATE TN **COUNTY** Putnam **AREA** Tennessee Technology University

SPECIES (NO.) Gray Rat Snake (*Pantherophis spiloides*) (1) **SEX** Unknown **AGE** Ad **WEIGHT** Unknown

CASE HISTORY: Two vials containing scales and four swabs from the thoracic and mandibular regions were submitted on September 24, 2014, by Chris Simpson of the Tennessee Wildlife Resources Agency for Snake Fungal Disease testing. The samples were processed on September 25, 2014.

FINAL DIAGNOSIS: Bacterial dermatitis; *Ophidiomyces ophiodiicola* not detected

COMMENTS: The fungus *Ophidiomyces ophiodiicola* was not detected by fungal culture or polymerase chain reaction from the samples submitted. While fungal organisms were identified upon microscopic examination of the scales, the morphology was not consistent with *O. ophiodiicola* and was not associated with inflammation. As the samples were very small, infection with *Ophidiomyces ophiodiicola* cannot be completely ruled out. Bacterial dermatitis could be due to previous trauma as well as abrupt changes in environmental conditions such as humidity or temperature outside the preferred optimal temperature zone for this species.

Mr. Simpson was notified of retrieval of the samples on September 25, 2014, by electronic mail.

WILDLIFE IMPLICATIONS: Snake Fungal Disease (SFD) is a dermatitis with deep fungal infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *Ophidiomyces ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Kevin D. Niedringhaus, BVetMed **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Simpson, Ratajczak, Sumners, Applegate, Piccirilli, Hatcher, AVIC

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC14-284
HISTO NUMBER W15-060

MICROSCOPIC FINDINGS: Multiple large colonies of basophilic coccobacilli are seen throughout the layers often within the stratum corneum associated with flakes of keratin and serocellular crusts. Small numbers of lymphocytes and heterophils are occasionally present within the surrounding epidermis and dermis. The scale contains linear tracts of brown staining granules on the external surface (interpreted as melanin). Rare fungal spores with morphology inconsistent with *Ophidiomyces ophiodiicola* are present within the sections Grocott's methanemine silver (GMS) staining.

MORPHOLOGIC DIAGNOSIS:

Scale: Mild, multifocal dermatitis with large bacterial colonies

MICROBIOLOGY: Swabs from both the ventrum and mandible as well as the scale from the mandible were plated on SDA fungal media and checked twice weekly for growth. Fungus from the plate with the mandibular swab was re-plated in an attempt to isolate a pure culture. Fungal growth did not morphologically appear consistent with *Ophidiomyces ophiodiicola*.

ANCILLARY TESTS: Polymerase chain reaction (PCR) did not detect the presence of *Ophidiomcyes ophiodiicola* from a swab of lesion on the ventrum. *O. ophiodiicola* was not detected using PCR of the fungus grown from the lesion on the mandible.

Appendix D

- 2015 Diagnostic Services Reports

DIAGNOSTIC SERVICES SECTION

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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FINAL REPORT

CASE NUMBER CC15-354
DATE RECEIVED August 10, 2015
DATE OF REPORT September 18, 2015

STATE TN **COUNTY** Dekalb **AREA** Indian Creek Center Hill Lake
SPECIES (NO.) Timber Rattlesnake (*Crotalus horridus*) (1) **SEX** M **AGE** 8 yrs **WEIGHT** 1792g

CASE HISTORY: A timber rattlesnake (PIT ID#025 026 610) captured in a drift fence trap on August 7, 2015, was noticed to have a skin lesion on its ventrum. Four swabs were collected by Mr. Danny L. Bryan of Cumberland University. They were submitted to SCWDS on behalf of Mr. Roger Applegate of the Tennessee Wildlife Resources Agency. They arrived August 10, 2015, and were processed the same day.

FINAL DIAGNOSIS: *Ophidiomyces ophidiicola* not detected

COMMENTS: The cause of this snake's skin lesion was not determined. The fungus that is associated with "snake fungal disease", *Ophidiomyces ophidiicola,* was not detected by polymerase chain reaction of the fungal colonies plated from the skin swabs. Histology of skin biopsies is recommended for evaluation of skin disease in snakes.

Mr. Applegate was notified of receipt of the samples on August 13, 2015, and a final diagnosis on September 15, 2015, by electronic mail.

MYCOLOGY FINDINGS: Four skin swabs were submitted for fungal culture and polymerase chain reaction (PCR) testing at the SCWDS laboratory. *Ophidiomyces ophiodiicola* was not detected in any sample following the initial PCR. Fungal cultures were monitored for 14 days, after which, three cultures with fungal growth consistent with *O. ophiodiicola* (A, C, D) were re-plated and monitored for an additional 14 days. At this point, one fungal culture had morphology similar to *O. ohiodiicola* (A). A sample of this colony was submitted to the SCWDS laboratory for PCR testing. *O. ophidiicola* was not detected.

WILDLIFE IMPLICATIONS: Undetermined.

PUBLIC HEALTH IMPLICATIONS: Undetermined.

DOMESTIC ANIMAL IMPLICATIONS: Undetermined.

DIAGNOSTICIAN Charlie Bahnson, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Hatcher, ADD

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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CASE NUMBER CC15-534
DATE RECEIVED October 7, 2015
DATE OF REPORT November 11, 2015

STATE TN **COUNTY** Cannon **AREA** Not recorded

SPECIES (NO.) Timber Rattlesnake (1) **SEX** Male **AGE** ~8 years **WEIGHT** 1.50kg

CASE HISTORY: Biopsies, swabs, and scale clips from an eight year old, male timber rattlesnake were submitted by Mr. Roger Applegate of the Tennessee Wildlife Resources Agency on October 5, 2015. These samples were collected on October 4, 2015, for snake fungal disease (SFD) surveillance testing. The samples were received on October 7, 2015, and were processed the same day. No tissue could be recovered from the vial labeled "punch biopsy sample."

The sample identification is described below:

Sample Number	Sample Description
TR-087-1	Punch Biopsy from dorsum
TR-087-2	Scale clip from dorsum
TR-087-3	Scale clip from dorsum
TR-087-4	Scale clip from dorsum
TR-087-5	Mass from ventral mandible
TR-087-6	Swab from the mouth
TR-087-7	Swab from mass under the left eye
TR-087-8	Swab from inside the mass from ventral mandible

FINAL DIAGNOSIS: Bacterial dermatitis; *Ophidiomyces ophiodiicola* not detected

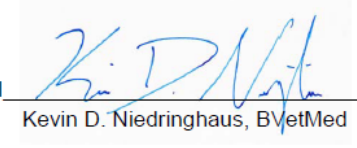
COMMENTS: *Ophidiomyces ophidiicola*, the fungus that has been associated with SFD, was not detected by culture or polymerase chain reaction in any of the samples, but this does not completely rule out the possibility of infection. Microscopic analysis of the mass under the chin revealed the presence of bacterial dermatitis and no fungal organisms were identified.

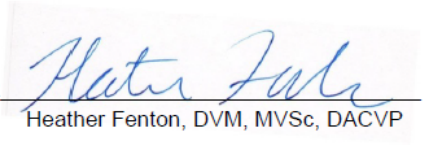
Mr. Applegate was notified of receipt of the samples on October 7, 2015, and of the final diagnosis on November 10, 2015, by electronic mail.

WILDLIFE IMPLICATIONS: Bacterial dermatitis is a common dermatological disease in many wild animals often as a secondary infection to injury or a primary pathogen. In snakes, this type of lesion is often referred to as 'blister disease" or "scale rot" and can also be associated with environmental factors. It is unlikely to be significant at the population level.

PUBLIC HEALTH IMPLICATIONS: None apparent.

DOMESTIC ANIMAL IMPLICATIONS: None apparent.

DIAGNOSTICIAN  Kevin D. Niedringhaus, BVetMed

SUPERVISOR  Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Hatcher, ADD

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC15-534
HISTO NUMBER W16-261

MICROSCOPIC FINDINGS:

Submandibular mass: The section is made up entirely of necrotic skin consisting of brightly eosinophilic, homogenous material containing pockets of Gram negative coccobacilli admixed with cellular and nuclear debris as well as degenerate heterophils. No fungal organisms are detected following Periodic Acid Schiff reaction.

MORPHOLOGIC DIAGNOSIS:

Submandibular mass: Severe, diffuse, focal necrotizing and heterophilic dermatitis

MICROBIOLOGY:

Samples TR-087-2 through TR-087-8 were plated on Sabouraud dextrose agar and allowed to incubate for three weeks. Samples from these cultures were submitted for *Ophidiomyces ophiodiicola (*Oo) testing. No Oo was detected in any sample by polymerase chain reaction (PCR).

Swabs from the mouth (TR-087-6), mass under the left eye (TR-087-7), and under the chin (TR-087-8), as well a sample of the submandibular mass (TR-087-087-5) and a scale from the back (TR-087-4) were submitted to the SCWDS laboratory for Oo testing. No Oo was detected in any of the samples by PCR.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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CASE NUMBER CC15-612
DATE RECEIVED November 11, 2015
DATE OF REPORT December 16, 2015

STATE TN **COUNTY** Dekalb **AREA** Edger Evins State Park

SPECIES (NO.) Black Racer (1) **SEX** Unknown **AGE** Not provided **WEIGHT** Not provided

CASE HISTORY: Skin biopsies and swabs collected from a black racer were submitted by Mr. Roger Applegate of Tennessee Wildlife Resource Agency and Dr. Danny Bryan of Cumberland University for snake fungal disease testing. The samples were taken November 10, 2015.

The samples were received November 11, 2015, and placed on a culture plate the same day.

FINAL DIAGNOSIS: Bacterial dermatitis; *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola* not detected

COMMENTS: The lesions observed in the skin are a non-specific finding and could have been due to trauma or difficulty shedding. Bacterial dermatitis is occasionally referred to as "blister rot." The fungal agent associated with Snake Fungal Disease (SFD), *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*, was not detected in this sample. This does not rule out the possibility of infection with this agent. Full thickness biopsy that includes a section of dermis is recommended for optimal diagnostic results. Ideally, the biopsy should include a region with normal and abnormal skin represented.

Dr. Bryan and Mr. Applegate were notified of receipt of the samples on November 15, 2015, by electronic mail. An update was provided on November 25, 2015, and a final diagnosis provided December 12, 2015, by electronic mail.

WILDLIFE IMPLICATIONS: Bacterial dermatitis is not uncommon in free-ranging snakes. Snake Fungal Disease (SFD) is an emerging disease associated with the newly described fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola* (Oo). A recent manuscript (Lorch et al. 2015) has established that Oo is the causative agent of SFD, although additional research continues.

Snake Fungal Disease is a dermatitis with deep fungal infection of the skin. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

Citation: Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS. 2015. Experimental infection of snakes with *Ophidiomyces ophiodiicola* causes pathological changes that typify snake fungal disease. mBio 6(6):e01534-15. doi:10.1128/mBio.01534-15.

PUBLIC HEALTH IMPLICATIONS: *Ophidiomyces ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: Bacterial dermatitis or "blister rot" is common in captive snakes. *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Mark G. Ruder, DVM, PhD **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Bryan, Hatcher, ADD

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS

FINAL REPORT

CASE NUMBER CC15-612
HISTO NUMBER W16-350

GROSS FINDINGS: Two skin swabs and a skin biopsy, approximately 1cm in maximum diameter, in formalin are received for diagnostic evaluation.

MICROSCOPIC FINDINGS:

Skin: Sections consist primarily of serocellular crusts are available. The epidermis and dermis cannot be easily evaluated. In some sections, small regions of dermis are present infiltrated by large numbers of lymphocytes. The epidermis is largely replaced by lymphocytes, heterophils, fibrin, and colonies of coccoid admixed with granular, yellow-to-brown foreign debris. No fungal organisms are observed with PAS staining.

MORPHOLOGIC DIAGNOSIS:

Skin: Moderate, subacute, focally extensive lymphocytic and heterophilic, fibrinonecrotizing dermatitis with intralesional bacteria and foreign material

MYCOLOGY: The skin swabs were submitted to the SCWDS Laboratory for fungal culture and polymerase chain reaction testing for *Ophidiomyces* spp. testing. Fungal culture and PCR were negative for *Ophidiomyces* spp.

Appendix E

- 2016 Diagnostic Services Reports

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC16-281
DATE RECEIVED May 27, 2016
DATE OF REPORT July 11, 2016

STATE TN **COUNTY** DeKalb **AREA** Edgar Evins State Park

SPECIES (NO.) Timber Rattlesnake (1) **SEX** Male **AGE** Adult **WEIGHT** 510.3 grams

CASE HISTORY: Two swabs and two scale clips from an adult, male timber rattlesnake (Tag #024882297) were submitted by Dr. Danny Bryan on behalf of Mr. Roger Applegate of the Tennessee Wildlife Resources Agency on May 24, 2016. These samples were taken from this snake for snake fungal disease surveillance on May 22, 2016. The samples were received by SCWDS on May 27, 2016, and were processed the same day.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* detected

COMMENTS: Fungal hyphae were observed in association with inflammation in remnants of scales in this snake. *Ophidiomyces ophiodiicola* was detected by fungal culture and real-time polymerase chain reaction. The extent of the inflammation cannot be evaluated in the samples provided.

Mr. Applegate and Dr. Bryan were notified of receipt of the samples on May 29, 2016, by electronic mail. They were provided the final diagnosis on July 11, 2016, by electronic mail.

WILDLIFE IMPLICATIONS: *Ophidiomyces ophiodiicola* is the causative agent of snake fungal disease (SFD), which is an emerging fungal disease of free-ranging snakes. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction, and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *Ophidiomyces ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Kevin D. Niedringhaus, BVetMed **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Bryan, Hatcher, TN ADD

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC16-281
HISTO NUMBER W16-726

GROSS FINDINGS: Not performed.

MICROSCOPIC FINDINGS:

Scale clips: Two sections of serocellullar crust are examined. The crust is composed of fibrin, amorphous eosinophilic material and degenerate heterophils. The cellular outlines are difficult to discern. Large clusters of primarily coccoid bacteria are present on the surface of the crust and are associated with pieces of plant material. Numerous fungal hyphae and spores are scattered throughout the necrotic tissue following periodic acid Schiff reaction (PAS). The hyphae are parallel-walled, approximately 4-7 microns in diameter, septate, and have right-angled branching. Small numbers of clavate to rectangular spores approximately 3 x 1 micron are present associated with fungal hyphae.

MORPHOLOGIC DIAGNOSIS:

Scale clips: Moderate, focally extensive, serocellular crust with intralesional fungal hyphae, arthrospores, and bacteria

MYCOLOGY: One scale clip and one swab were plated on Rapid Sporulation Media for fungal culture. The fungus isolated is morphologically consistent with *Ophidiomyces ophiodiicola*.

One scale clip and one swab were submitted to the SCWDS laboratory for snake fungal disease testing. *Ophidiomyces ophiodiicola* was detected in both samples by real-time polymerase chain reaction.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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CASE NUMBER CC16-362
DATE RECEIVED July 22, 2016
DATE OF REPORT August 15, 2016

STATE TN **COUNTY** DeKalb **AREA** Edgar Evins State Park

SPECIES (NO.) Timber Rattlesnake (1) **SEX** Female **AGE** ~6 years **WEIGHT** Not recorded

CASE HISTORY: Five swabs and two scale clips from a female timber rattlesnake were submitted by Dr. Danny Bryan on behalf of Mr. Roger Applegate of the Tennessee Wildlife Resources Agency on July 21, 2016. These samples were collected for snake fungal disease surveillance on July 20, 2016. The snake reportedly had three small lesions on the skin. The samples were received on July 22, 2016, and were processed the same day.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* detected

COMMENTS: Fungal hyphae were observed microscopically in the scale clips provided and were associated with inflammation. *Ophidiomyces ophiodiicola* was detected in two swabs by real-time polymerase chain reaction. The severity of the infection or extent of the lesions cannot be evaluated in scale clips.

Dr. Bryan and Mr. Applegate were notified of receipt of the samples on July 22, 2016, by electronic mail. They were provided the final diagnosis on August 13, 2016, by electronic mail.

WILDLIFE IMPLICATIONS: *Ophidiomyces ophiodiicola* is the causative agent of snake fungal disease (SFD), which is an emerging fungal disease of free-ranging snakes. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction, and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes previously were reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor)*, rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*), have been diagnosed with SFD. As of this time, this disease is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: *Ophidiomyces ophiodiicola* grows at approximately 20°C and is not thought to be infectious to humans.

DOMESTIC ANIMAL IMPLICATIONS: *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

DIAGNOSTICIAN Kevin D. Niedringhaus, BVetMed **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Bryan, Hatcher, TN ADD

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS

FINAL REPORT

CASE NUMBER CC16-362
HISTO NUMBER W17-029

MICROSCOPIC FINDINGS:

Scale clips: Five scale clips are examined. All scales are composed of keratin admixed with amorphous eosinophilic material that lacks cellular detail, degenerate heterophils, and cellular debris. Abundant coccoid bacteria are present on the surface. Faint outlines of fungal hyphae are present within the deeper areas of the scales that stain with periodic acid Schiff reaction. The hyphae are approximately 5-7 microns in diameter, have parallel walls and rare dichotomous, right-angled branches.

MORPHOLOGIC DIAGNOSIS:

Scale clip: Serocellular crust with intralesional fungal hyphae and bacterial colonies

MYCOLOGY: Two swabs were submitted to the SCWDS laboratory for *Ophidiomyces ophiodiicola* (Oo) testing. The fungus that causes snake fungal disease, Oo, was detected by real-time polymerase chain reaction.

Two swabs were plated on rapid sporulation media. The fungus isolated from one swab is not morphologically similar to Oo. No fungi were isolated from the other swab.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
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ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC16-378
DATE RECEIVED July 28, 2016
DATE OF REPORT September 7, 2016

STATE TN **COUNTY** DeKalb **AREA** Edgar Evins State Park

SPECIES (NO.) Timber Rattlesnake (1) **SEX** Male **AGE** 9 years **WEIGHT** 1361 grams

CASE HISTORY: A timber rattlesnake (AVID 025049376) was captured on July 26, 2016. At the time of capture, lesions were noted on the left eye and left ventrum and four swabs were collected. They were submitted by Mr. Roger Applegate of the Tennessee Wildlife Resources Agency. They were received at SCWDS on July 28, 2016.

FINAL DIAGNOSIS: *Ophidiomyces ophiodiicola* detected

COMMENTS: The fungus that causes snake fungal disease (SFD), *Ophidiomyces ophidiicola,* was detected in the swab from the body by real-time polymerase chain reaction. It was not detected in the other three swabs, but this does not rule out the presence of the fungus at these locations.

Mr. Applegate was notified of receipt of the samples on July 28, 2016, and provided a final diagnosis on September 6, 2016, by electronic mail.

WILDLIFE IMPLICATIONS: Snake fungal disease (SFD) is an emerging fungal disease of free-ranging snakes caused by infection with the fungus *Ophidiomyces ophiodiicola*. Clinical signs include scabs, crusty scales, abnormal molting, cloudiness of the eyes, skin ulcers, fang destruction, and subcutaneous nodules. Lesions on the head are reported most often, but lesions can occur anywhere on the body. Although sporadic accounts of fungal dermatitis in free-ranging snakes were previously reported, the number of cases has increased since 2006. Multiple species, including the northern water snake (*Nerodia sipedon),* eastern racer (*Coluber constrictor*), rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrururs miliarius*), and milk snake (*Lampropeltis Triangulum*) have been diagnosed with SFD. At this time, SFD is not known to infect reptiles other than snakes. Disease has been found in individual animals, but may contribute to population declines, particularly in threatened species.

PUBLIC HEALTH IMPLICATIONS: None apparent.

DOMESTIC ANIMAL IMPLICATIONS: *Ophidiomyces ophiodiicola* should not pose a risk to domestic mammalian or avian species. However, snakes kept as pets or on display may potentially be at risk for infection.

MICROBIOLOGY: Four swabs designated as "from eye," "from the body," "from the mouth," and "cotton swab" were submitted to the SCWDS Microbiology Laboratory. *Ophidiomyces ophiodiicola* was detected in the swab from the body by real-time polymerase chain reaction. It was not detected in the other three swabs.

DIAGNOSTICIAN Charlie Bahnson, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Applegate, Bryan, Hatcher, TN ADD

Appendix F

- 2017 Diagnostic Services Reports

NATIONAL WILDLIFE HEALTH CENTER

6006 Schroeder Road
Madison, Wisconsin 53711-6223
608-270-2400 (FAX 608-270-2415)

DIAGNOSTIC SERVICES CASE REPORT

Case: 28376
Epizoo:

Final Report 11/8/2017

Legal ☐ **Declassified** ☐ **INV#:**

Submitter:
Donald Walker
Tennessee Technological University
Box 5063
1 William L Jones Dr
Cookeville, TN 38505

Date Submitted: 11/2/2017

Specimen description/Identification/Location:

ACC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER's ID	COUNTY	STATE
001	Snake, Timber Rattlesnake	SWAB, NOS	TST2		White	TN
002	Snake, Copperhead	SWAB, NOS	AGCO		De Kalb	TN
003	Snake, Timber Rattlesnake	SWAB, NOS	TST3		Cumberland	TN
004	Snake, Timber Rattlesnake	SWAB, NOS	TST5		Morgan	TN
005	Snake, Timber Rattlesnake	SWAB, NOS	TST6		Morgan	TN
006	Snake, Timber Rattlesnake	SWAB, NOS	TST4		Morgan	TN
007	Snake, Eastern Milk	SWAB, NOS	MISNBS1		Grainger	TN
008	Snake, Eastern Milk	SWAB, NOS	MISNTC1		Claiborne	TN
009	Snake, Eastern Milk	SWAB, NOS	MISNTC2		Claiborne	TN
010	Snake, Eastern Milk	SWAB, NOS	MISNNC1		Campbell	TN
011	Snake, Eastern Racer	SWAB, NOS	EARATC1		Claiborne	TN
012	Snake, Eastern Racer	SWAB, NOS	EARATC2		Claiborne	TN
013	Snake, Rat	SWAB, NOS	RASNBS1		Grainger	TN
014	Snake, Unidentified hognose	SWAB, NOS	STP1635H		De Kalb	TN
015	Snake, Unidentified hognose	SWAB, NOS	STP1635c		De Kalb	TN
016	Snake, Black Kingsnake	SWAB, NOS	BLKIBS1		Grainger	TN
017	Snake, Corn	SWAB, NOS	COSNLC1		Greene	TN
018	Snake, Eastern Racer	TISSUE, SWAB	EARALC1		Greene	TN
019	Snake, Rat	TISSUE, SWAB	RASNLC1		Greene	TN
020	Snake, Eastern Racer	SWAB, NOS	EARALC2		Greene	TN

Diagnosis:

1. Suspect Snake Fungal Disease (Acc. 018, 019)
2. Positive for Ophidiomyces ophiodiicola by PCR (Acc. 004, 007-009, 012, 014-020)
3. Negative for Ophidiomyces ophiodiicola by PCR (Acc. 001-003, 005-006, 010-011, 013)

Event History:

Swabs and scale clips from 20 snakes sampled from 7 TN counties between April - October 2017 were submitted as part of the CompSWG SFD project. Eighteen of 20 snakes swabbed are suspect asymptomatic carriers of Ophidiomyces ophiodiicola, the causative agent of snake fungal disease. Two snakes (EARALC1 and RASNLC1) had suspected skin lesions similar to those found on individuals with snake fungal disease; scale clips were provided. The scale clips have been frozen at -20C for about three months.

White County:
Timber Rattlesnake (TST2): Swab collected 7/8/17.
Copperhead (AGCO): Found after a prescribed burn and swab collected 4/12/17.
Both samples were collected from Virgin Falls WMA.

Claiborne County:
Eastern Racer (EARATC1) [36.53128 -83.84817]: Swab collected 6/7/17.
Eastern Racer (EARATC2) [36.53128 -83.84817]: Swab collected 6/7/17.
Milksnake (MISNTC1) [36.53453 -83.84726]: Swab collected 6/7/17.
Milksnake (MISNTC2) [36.53128 -83.84817]: Swab collected 6/7/17.

Campbell County:
Milksnake (MISNNC1) [36.36338 -84.26048]: Swab collected 5/29/17.

Case: 28376 **Final Report** 11/8/2017
Epizoo:

Legal ☐ Declassified ☐ INV#:

DeKalb County:
Eastern Hog-nosed Snake (STP1635H) [36.08628 -85.81171]: Swab collected 10/5/17.
Eastern Hog-nosed Snake (STP1635C) [36.08628 -85.81171]: Swab collected 10/5/17.

Grainger County:
Western Rat Snake (RASNBS1) [36.20873 -83.5652]: Swab collected 6/13/17.
Eastern Kingsnake (BLKIBS1) [36.20432 -83.56297]: Swab collected 5/22/17.
Milksnake (MISINBS1) [36.20432 -83.56297]; Swab collected 5/22/17.

Greene County:
Corn Snake (COSNLC1) [36.1605 -83.08964]: Swab collected 5/25/17.
Eastern Racer (EARALC1) [36.1605 -83.08964]: Swab and scale clip collected 5/25/17.
Eastern Racer (EARALC2) [36.16199 -83.0819]: Swab collected 5/25/17
Western Ratsnake (RASNLC1) [36.16199 -83.0819]: Swab and scale clip collected 5/25/17.

Morgan County:
Timber Rattlesnake (TST3): Swab collected 7/9/17.
Timber Rattlesnake (TST4): Swab collected 7/31/17.
Timber Rattlesnake (TST5): Swab collected 8/13/17.
Timber Rattlesnake (TST6): Swab collected 8/27/17.
All samples found in Catoosa WMA. Specimens were found on the road during diurnal surveys.

Comment:
A subset of samples collected from snakes from this case tested positive for Ophidiomyces, the causative agent of snake fungal disease (SFD), by real-time PCR.* Note that there was a disparity in results between the swab and tissue sample for Acc. 19. The swab sample yielded a weak positive (near the detection limit of the assay) whereas the tissue sample tested negative. This is likely attributed to a low amount of Ophidiomyces DNA being present in the samples. Similarly low amounts of DNA were detected in Acc. 004, 009, 012, and 020. Detection of Ophidiomyces DNA alone does not indicate that a snake has SFD; however, because Acc. 018 and 019 were observed with concurrent skin lesions suggestive of SFD, these snakes are considered suspect positive for SFD. A definitive diagnosis of SFD requires histological examination of skin which could not be performed in this case due to the nature of the samples.

*Note the lack of a positive result by PCR does not definitively indicate the absence of the organism. PCR may not detect the organism if it is at very low abundance in the sample.

Wildlife and Domestic Animal Significance: Snake fungal disease (SFD) is a fungal infection of snakes caused by the pathogen *Ophidiomyces ophiodiicola*. Examinations of snakes with SFD indicate that the fungus invades deeply into the epidermis and dermis, hence molting may not rid the animal of infection despite temporary resolution of clinical signs. The significance of SFD in free-ranging snakes is currently under investigation, and the threat that SFD poses is believed to vary between snake populations. As the name of the disease implies, SFD is only known to affect snakes.

Human Health Considerations: None

Disease Control and Biosecurity: Wear clean disposable gloves when handling sick or dead snakes. Clean supplies and field equipment with soap and water followed by disinfection with a 10% bleach solution (9 part water, 1 part bleach) between animals and sites. When SFD is already known to occur in a region, snakes whose skin lesions appear to resolve with supportive care and/or antifungal therapy may be candidates for release at their capture site, but these individuals should not be released in an area where the disease has not been previously as it is not known if treated snakes may still harbor viable fungus.

Please continue to monitor this mortality event and provide periodic updates to NWHC Epidemiology Team throughout the course of the event as additional considerations and further investigation may be warranted.

Case: 28376
Epizoo:

Final Report 11/8/2017

Legal ☐ Declassified ☐ INV#:

Jeffrey M. Lorch Ph.D.
Diagnostic Microbiologist
Phone: 608-270-2420 **Email:** jlorch@usgs.gov

Diagnostic findings may not be used for publication without the pathologist's knowledge and consent.

Copies To:

ANNE STENGLE
Univ of Mass/Dept Env Conserv/Holdsworth NatResCenter, 160 Holdsworth Way, Box 34210, Amherst, MA 01003-4210

ROGER APPLEGATE
Tennessee Wildlife Resources Agency- Region 2, PO Box 40747, Ellington Agricultural Center, Nashville, TN 37204

This is a Report for your submission to the National Wildlife Health Center.

For consultation regarding diagnostic findings or laboratory testing and results, please contact the pathologist. Contact information can be found underneath the signature line on this report.

For consultation on the significance of this disease to wildlife populations in your area, assistance with disease control and response, or to report field updates (numbers and species affected, geographical distribution, end date, etc.), please contact an NWHC epidemiologist at NWHC-epi@usgs.gov or 608-270-2480.