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### DoD SNAKE FUNGAL DISEASE SURVEY: NATURAL RESOURCE MANAGER TRAINING AND DATA COLLECTION

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## TABLE OF CONTENTS

<b>Abstract</b> .....	<b>6</b>
<b>Introduction</b> .....	<b>7</b>
<b>Materials and Methods</b> .....	<b>8</b>
<b>Results</b> .....	<b>10</b>
<b>Discussion</b> .....	<b>11</b>
<b>Benefits to Military</b> .....	<b>13</b>
<b>Management Implications</b> .....	<b>14</b>
<b>Literature Cited</b> .....	<b>15</b>
<b>Table 1</b> .....	<b>19</b>
<i>Military installations, by state, where snakes were sampled in 2018 for detection of ophidiomycosis</i>	
<b>Table 2</b> .....	<b>21</b>
<i>Sample sizes by species for snakes assayed for <i>Ophidiomyces ophiodiicola</i> on military installations from 2018</i>	
<b>Table 3</b> .....	<b>23</b>
<i>Free-ranging snake species detected with <i>Ophidiomyces ophiodiicola</i> for the first time during surveillance conducted on military installations, 2018</i>	
<b>Table 4</b> .....	<b>24</b>
<i>AIC rankings of the top 5 logistic regression models predicting ophidiomycosis classification in species of snakes sampled on military installations in 2018. Sp = species, A = age class, S = state</i>	
<b>Table 5</b> .....	<b>24</b>
<i>Odds ratios for states that were significantly associated with ophidiomycosis sampled in different states on military installations in 2018</i>	
<b>Table 6</b> .....	<b>25</b>
<i>AIC rankings of the top 5 logistic regression models predicting ophidiomycosis classification detection in snake species sampled on military installations in 2018. Sp = species, A = age class, I = installation</i>	
<b>Table 7</b> .....	<b>25</b>

*Significant predictors of ophidiomycosis classification in species of snakes sampled on military installations in 2018. A positive estimate is associated with an increased probability of ophidiomycosis.*

<b>Table 8</b> .....	<b>26</b>
<i>Prevalence of possible ophidiomycosis (clinical signs in the absence of Ophidiomyces qpcr positive result) in species of snakes sampled on military installations in 2018</i>	
<b>Table 9</b> .....	<b>27</b>
<i>Prevalence percentages of Ophidiomycosis classifications in species of snakes sampled on military installations in 2018</i>	
<b>Supplemental table 1</b> .....	<b>28</b>
<i>List of genbank numbers for gene sequences of the cytochrome b gene in snakes sampled in 2018 at DoD military installations for ophidiomycosis. Sequences were used to generate a phylogenetic tree adapted from Figueroa et al. 2016</i>	
<b>Figure 1</b> .....	<b>30</b>
<i>Spatial distribution of Ophidiomyces ophiodiicola detection in snakes on military installations sampled in 2018. White = states not sampled, light grey = states with no detection of O. ophiodiicola, dark grey = states detected with O. ophiodiicola white astericks indicates a state/territory identified with O. ophiodiicola the first time in this study</i>	
<b>Figure 2</b> .....	<b>31</b>
<i>Spatial distribution and prevalence of ophidiomycosis in snakes on military installations sampled in 2018. Negative = no clinical signs and no qPCR detection of Ophidiomyces ophiodiicola; present = qPCR positive and no clinical signs; possible = clinical signs present and no qPCR detection; apparent = qPCR detection and clinical signs</i>	
<b>Figure 3</b> .....	<b>32</b>
<i>Phylogenetic analysis of snake species detected with Ophidiomyces ophiodiicola using qPCR from sampling on military installations in 2018. Green = 0% prevalence; yellow = 0-9.9%; light orange = 10-24.9%; dark orange = 25-49.9%; red = &gt;50%.</i>	

**Figure 4** ..... **33**  
*Photographs of swab sampling (A,B) of snakes for detection of Ophidiomyces on military installations in 2018. A ophidiomycosis (Snake fungal disease) lesion in Crotalus oreganus helleri (C), Pituophis melanoleucus (D), Chilabothrus inornatus (E), and Pantherophis spiloides (F).*

**Appendix 1** ..... **34**  
*Snake sampling protocol sent to each participating department of defense installation*

**Appendix 2.** ..... **36**  
*Snake sampling data sheet sent to each participating department of defense installation*

## **LIST OF ABBREVIATIONS**

qPCR	Quantitative PCR
DoD	Department of Defense
WEL	Wildlife Epidemiology Lab
SVL	Snout to vent length
SFD	Snake fungal disease
DNA	Deoxyribonucleic acid
AIC	Akaike Information Criterion
NMFWA	National Military Fish and Wildlife Association

## ABSTRACT

Snakes play essential roles as both predators and prey in the ecosystems of Department of Defense (DoD) lands. Ophidiomycosis (formerly referred to as Snake Fungal Disease, SFD), an emergent pathogen on the North American landscape caused by the fungal pathogen *Ophidiomyces ophiodiicola*, poses a threat to snake population health and stability. It has been documented in over 15 genera of wild and captive snakes and infection causes a wide range of clinical signs, from difficulty shedding to crusts and ulcers on the head and body, and even death in some cases. We set out to develop outreach materials and sampling protocols, conduct a training session for military natural resource managers to enable them to sample for SFD on their respective installations, and test snakes sampled on DoD installations for *O. ophiodiicola* DNA using quantitative PCR (qPCR). Sampling kits were sent to 68 installations, of which 56 returned swabs, for a 82% participation rate. A total of 657 individuals representing 58 species in 31 states were observed and tested for *Ophidiomyces*. Twenty-five species from 19 states/territories were detected with *O. ophiodiicola* DNA, including the first reports of the pathogen in snakes in Idaho, Oklahoma, and Puerto Rico. Apparent ophidiomycosis (lesions and *O. ophiodiicola* qPCR positive) was observed in 49 individuals, *O. ophiodiicola* qPCR positive was detected in 64 individuals in the absence of clinical signs, 82 snakes had possible ophidiomycosis (lesions but *O. ophiodiicola* qPCR negative), and 462 were qPCR negative and lacked lesions. Multinomial multivariable logistic regression identified adults as having 2.38 greater odds of being diagnosed with ophidiomycosis. Snakes from Georgia, Massachusetts, Pennsylvania, and Virginia all had greater odds of detection of ophidiomycosis, while snakes from Idaho were less likely to be detected with ophidiomycosis. The results of this survey indicate that this pathogen is endemic in certain areas of the country (eastern US), but also identified new sites that could represent emergence or improved detection of endemic sites. The direct mortality of snakes with ophidiomycosis is unknown from this study, but the presence of numerous individuals with clinical disease warrants further investigation and possible conservation action.

## INTRODUCTION

Global landscapes have experienced unprecedented changes in the last 100 years, and many habitats no longer resemble the ecosystems in which species evolved. These landscape changes are associated with population and species declines via habitat destruction, climate change, and infectious disease, among other factors. Deteriorating wildlife health further threatens the sustainability of natural resource management, the prevention of human disease, and the success of efforts to conserve biodiversity. Natural resources on military lands support a large percentage of America's endangered habitats and species (Stein et al. 2008). Furthermore, the amphibian and reptile species confirmed present on Department of Defense (DoD) sites represent 66% of the total native species documented in the continental U.S. (Petersen et al., 2018). As a result, the DoD has implemented an ecosystem management approach to maintain and/or restore biological diversity and use land and water resources on its properties to ensure sustainability of military readiness (Benton et al. 2008), and also manages herpetofauna under guidance from a comprehensive strategic plan (Lovich et al. 2015). It is therefore the duty of military natural resource personnel to focus on the military mission, think regionally, and rely on the best available science to form partnerships and balance the impacts of training with biodiversity conservation.

Many native snake populations are at risk due to global landscape changes. Snakes fulfill numerous important functions for maintaining healthy ecosystems and subsequently promoting global health. As generalist predators, they control populations of small mammals and therefore aid in controlling the spread of zoonotic diseases such as hantavirus and Lyme disease (Ostfeld and Holt, 2004). It was observed that Timber Rattlesnakes' small mammal consumption was responsible for the removal of 2500-4500 ticks per year, which would reduce the incidence of Lyme disease in the areas where these snakes live (Kabay, Caruso, and Lips, 2013). In addition, snakes are crucial to maintaining food chains in many ecosystems, as they serve as prey for higher level predators, including birds and other snakes. Healthy snake populations, therefore, promote overall biodiversity, which is integral for maximizing ecosystem health and productivity (Grace *et al.*, 2016). Military lands are home to 131 snake species, several currently either listed or candidates for listing as threatened or endangered by the USFWS (e.g. Eastern Indigo Snake, Louisiana Pinesnake, Black Pinesnake, Giant Gartersnake, Eastern Massasauga; Petersen et al., 2018). Therefore, understanding potential threats to these species is of conservation interest and may have implications for military activities.

Sustainability and success of conservation efforts is threatened by diseases, including white nose syndrome (Blehert et al., 2009), ranavirus (Johnson et al., 2008), and chytridiomycosis (Skeratt et al., 2007). Investigating pathogen occurrence in amphibians on DoD lands has aided in conservation missions (Crawford et al., 2017; Petersen et al., 2016; Lannoo et al., 2011), but to date, efforts have not been made to investigate the health of snakes. Ophidiomycosis (formerly known as Snake Fungal Disease; SFD) is an emerging infectious disease of wild and captive

snakes that poses a potential threat to snake biodiversity. Experimental infection studies have determined that the disease is caused by the keratophilic fungus *Ophidiomyces ophiodiicola* (Allender *et al.*, 2015a; Lorch *et al.*, 2015) and the disease has been observed in more than 30 species of snakes in the United States and Europe (Allender *et al.*, 2015c; Burbrink *et al.*, 2017; Franklino *et al.*, 2017; Lorch *et al.*, 2016). Interest in monitoring the prevalence of SFD has increased since its implication in the declines of populations of Timber Rattlesnakes (*Crotalus horridus*; Clark *et al.* 2011) and Eastern Massasaugas (*Sistrurus catenatus*; Allender *et al.* 2016). Clinical signs of SFD include accelerated ecdysis cycles, epidermal flaking and crusting, displaced and/or discolored scales, granulomas, nodules, and swelling or disfiguration of infected tissues (Baker *et al.*, 2019).

Molecular diagnostics are increasingly important tools for understanding the epidemiology of infectious diseases of wildlife, specifically reptiles (Archer *et al.*, 2017; O’Dea *et al.*, 2016; Pontremoli *et al.*, 2018). The significant variation in the presentation of ophidiomycosis across species and between individual snakes (Lorch *et al.*, 2016) necessitates highly sensitive, non-invasive methods for detection. Clinical signs have been shown to be a good predictor of *O. ophiodiicola* detection (Allender *et al.*, 2016; McKenzie *et al.*, 2018), but simple swabbing techniques may have a high false negative rate (Hileman *et al.*, 2018). However, swabbing was found to be in good agreement with scale clips and is less invasive which may reduce disease transmission compared to biopsies/scale clips (McKenzie *et al.*, 2018). Recently, a case definition for ophidiomycosis was published that establishes the specific criteria for various ophidiomycosis categories, utilizing the results of qPCR from swab samples in combination with clinical signs (Baker *et al.*, 2019). The inclusion of clinical signs and a scoring system has been established for Pygmy Rattlesnakes (*Sistrurus miliaris*: McCoy *et al.*, 2017), but its utility across other species is needed.

Thus, important baseline health data is lacking for snakes on DoD sites nationwide. Characterizing the prevalence of ophidiomycosis and its causative agent, a pathogen with conservation significance, will allow assessment of critical spatial and taxonomic variability to minimize the impact of this pathogen on snake populations, particularly of imperiled species. Thus, our project specifically set out to: 1. Determine the occurrence of *O. ophiodiicola* on DoD lands across North America and its territories; 2. Determine the significant demographic, spatial, or taxonomic associations with *Ophidiomyces*; and 3. Make recommendations that aim for the prevention of negative impacts to military readiness as a result of degrading ecosystem health in the presence of ophidiomycosis.

## MATERIALS AND METHODS

**STUDY SITES**—*O. ophiodiicola* sampling kits were provided to 68 military installations, and samples were received from 56 (Table 1; Figure 2). The sites ranged across the US and a number of habitat types.



**FIELD SAMPLE COLLECTION**– Surveys of snakes for *O. ophiodiicola* began on 1 April 2018 and samples were collected through 31 December 2018 (8 months). Upon capture, all snakes were visually inspected for skin lesions, scabs, or other areas that may indicate an ophidiomycosis infection (Appendix 1). Ophidiomycosis status was categorized based on a recently described case definition (Baker et al., 2019). Categories included: 1) Negative (no clinical signs or qPCR detection of *O. ophiodiicola* DNA), 2) *Ophidiomyces* present (qPCR detection in absence of clinical signs), 3) Possible ophidiomycosis (presence of clinical signs in absence of qPCR detection), and 4) Apparent ophidiomycosis (presence of clinical signs and qPCR detection).

After inspection, swab samples were collected from all individuals using sterile cotton-tipped applicators which were provided in the sampling kits. Sterile handling procedures (i.e., a combination of rubber gloves and sanitizing hands and processing equipment with an alcohol or bleach solution) were used while collecting samples (Rzadkowska et al., 2016). A link to a training video (<https://www.youtube.com/watch?v=PxuPCMppeIY&feature=youtu.be>) and a detailed written protocol (Appendix 1) were sent to each collaborator to standardize sample collection. For snakes that had no apparent lesions on the skin, two whole-body swab samples were collected by making eight passes along the body with each swab, a slight modification to previously reported method (Hileman et al., 2018). If an individual had skin lesions that could indicate ophidiomycosis, additional swabs directly from each affected area(s) were collected, with a maximum of four lesion swabs from each individual. All swab samples were placed in separate 2.0 ml Eppendorf tubes and frozen within 2 hours until shipment. Demographic characteristics (species, sex, age class) were recorded for each individual on a standardized data sheet (Appendix 2).

**QUANTITATIVE PCR**–DNA extraction and quantitative PCR amplification (qPCR) were performed on swabs as previously reported (Allender et al., 2015a). DNA extraction followed the manufacturer’s recommendations with the addition of an incubation at 37°C with 25U of lyticase prior to the lysis step. Following DNA extraction, each sample was assessed for DNA quantity (measured in ng/μl) and quality (using the ratio of absorbance at 260 nm to 280 nm) using spectrophotometry (Nanodrop, ThermoFisher Scientific). qPCR was performed in triplicate on a QuantStudio3 real time thermocycler. Samples were considered positive if replicates had a lower mean cycle threshold ( $C_t$ ) value than the lowest detected standard dilution. Copies per reaction were standardized to the total quantity of DNA in the sample by dividing the mean copies/μl for each sample by the DNA concentration, as determined by spectrophotometry.

**STATISTICAL ANALYSIS**–Prevalence of each ophidiomycosis category was estimated by calculating the 95% binomial confidence interval (Wilson, 1927) in total and by sex, age class, installation, and month. Fisher’s Exact test was used to test associations between ophidiomycosis status with each demographic characteristic. Variables with a significance of <0.1 were included in a series of logistic regression models to evaluate the effects of independent variables (species,

sex, age class, installation, month) on the output variable (ophidiomycosis category). Dummy variables in the models were established for each categorical variable: species (*Agkistrodon contortrix*), age class (adult), state (Alabama), and installation (Arnold Air Force Base). Since installation and state overlapped, separate models were run that included each of those variables; both variables were not included in any model set. Next, an information theoretic approach was used to determine which model from the candidate set performed best using the AICcmodavg package (Mazerole, 2017). All factors and 2-way interactions were included. Higher order interactions were not pursued due to sample size constraints. Odds ratios (OR) were then calculated for significant predictors using epiDisplay package (Chongsuvivatwong, 2018). Normality of standardized DNA copies was assessed using the Shapiro-Wilks test. Mean and 95% CI were then calculated and compared between species using a Kruskal Wallis test. Ophidiomycosis prevalence data was visually evaluated based on snake phylogenetic relatedness at the cytochrome b gene loci as previously described (Figueroa et al., 2016; Supplemental Table 1) using Geneious (BioMatters, Inc, Newark, NJ 07102). For phylogenetic analysis, subspecies were not evaluated separately. Statistical significance was assessed at  $\alpha=0.05$  and all statistical analyses were conducted using R (R Development Core Team, 2016; SPSS ver. 24, Chicago, IL 60606; MedCalc).

## RESULTS

**GENERAL SURVEY RESULTS**— Over 4,500 qPCR reactions for 1460 swabs were assayed from 657 individual snakes. Snakes were sampled from 56 military installations from 31 states (Table 1; Figure 1). Snakes sampled represented 58 species (Table 2). There were 430 adults, 121 juveniles, and 106 snakes of unknown age sampled. Some samples (n=242) arrived at room temperature, rather than being kept cold during shipment, due to a range of shipping inequities. Samples that arrived warm (mean: 4.35 ng/ $\mu$ l) had a lower DNA concentration than samples that arrived cold (mean: 6.47 ng/ $\mu$ l) ( $p<0.0001$ ). There was no difference in purity of DNA between samples that arrived warm or cold ( $p=0.597$ ). Standardized fungal copy number in positive samples was non-significantly higher in samples that arrived cold (mean: 456.19 copies/ng DNA) than warm (mean: 168.08 copies/ng DNA) ( $p=0.293$ ). Positive samples (mean: 8.76 ng/ $\mu$ l) had a higher concentration of DNA than negative samples (mean: 5.30 ng/ $\mu$ l) ( $p=0.013$ ), but there was no difference in purity ( $p=0.694$ ).

**OPHIDIOMYCES DETECTION AND OPHIDIOMYCOSIS CLASSIFICATION**— Individuals were captured once each and swabbed from 1 to 9 times. Skin lesions were observed in 131 individuals for an overall prevalence of 19.9%. *Ophidiomyces ophiodiicola* DNA was detected in samples from 113 snakes for a prevalence of 17.2% (95% CI: 14.4 – 20.3%). Nineteen states/territories were detected with *O. ophiodiicola* DNA, including for the first time Idaho, Oklahoma, and Puerto Rico (Figure 1). Forty-nine (43.4%) of the qPCR positive individuals had skin lesions. Skin lesions were significantly associated with a qPCR positive result ( $p<0.0001$ ).

Twenty-three of the sampled species had qPCR positive results and this is the first reported occurrence of *O. ophiodiicola* in four of those species (Table 3). All four categories of ophidiomycosis were represented in this study. Most animals were ophidiomycosis negative (n=462), with *Ophidiomyces* present (n=64), possible ophidiomycosis (n=82; Table 8), and apparent ophidiomycosis (n=49) occurring less frequently (Figure 2). There was no difference in standardized *Ophidiomyces* copy number between installations (p=0.750). There was no clear association with snake phylogenetic relatedness and detection of *O. ophiodiicola*, but the genera *Nerodia*, *Pantherophis*, and *Drymarchon* had the highest prevalence (Figure 3).

**LOGISTIC REGRESSION MODELS**– To increase the predictive power of the models, species with less than 5 individuals represented were removed. Then, a series of multinomial multivariable logistic regression models were fit for ophidiomycosis status (4 output categories). The final data set for the model included 595 individuals representing 394 adults, 101 juveniles, and 100 snakes of unknown age. There were 419 negative, 55 *Ophidiomyces* present, 78 ophidiomycosis possible, and 43 apparent ophidiomycosis individuals. The final model with state included the following variables: state (p=2.2 x 10<sup>-16</sup>) and age (p=0.003) (Table 4). The significant positive predictors included the states Georgia, Massachusetts, Pennsylvania, and Virginia (Table 5). The final model with installation included the following variables: installation (p=2.2 x 10<sup>-16</sup>) and age (p=0.003) (Table 6). The significant negative predictor was juvenile age class (Table 7). Juveniles were 2.38 (95% CI: 1.39-4.17, p=0.002) times less likely to have ophidiomycosis than adults in both models (Table 9).

## DISCUSSION

Ophidiomycosis has potentially serious consequences for the success of snake conservation efforts in North America (Allender et al., 2015c; Lorch et al., 2016). We detected *O. ophiodiicola* DNA in 23 species and four of those have not been previously reported in the literature. While ophidiomycosis is associated almost exclusively, to date, with skin lesions, infections may be associated with systemic infection (Dolinski et al., 2015; Robertson et al., 2016). Skin lesions were not uncommon in this study, and somewhat surprisingly, there were several animals with skin lesions in which *Ophidiomyces* DNA was not detected (possible ophidiomycosis). This may signify the difficulty in sampling for this disease (low DNA quantity on the skin or fungi in tissues deeper than the epidermis), the similar appearance of ophidiomycosis lesions to non-infectious skin disease such as trauma, the presence of another pathogen causing similar skin lesions, or false negative result due to sample collection or handling. It is interesting to note that the negative predictive value of skin lesions in detecting *O. ophiodiicola* DNA was 84.9%, while the positive predictive value was only 43.4%. This should give biologists and veterinarians hesitation to assign causation by *O. ophiodiicola* to animals with skin lesions. Conversely, the negative predictive value of clinical signs is better, making it more reliable to use the lack of clinical signs to eliminate a diagnosis of apparent ophidiomycosis. This is consistent with previous literature that showed that the rate of false

negatives is nearly 10 times higher in animals without lesions than individuals with lesions (Hileman et al., 2018). The current sampling recommendation to reduce the false negative rate is to repeatedly and firmly swab along the entire surface of the skin eight times along the length of the snake (Hileman et al., 2018).

No species had a significantly higher prevalence of ophidiomycosis, but the phylogenetic analysis showed clustering of positive cases among some species of *Nerodia*, *Pantherophis*, and *Drymarchon*, while negative cases were clustered in genera *Liodytes*, *Virginiae*, *Storeria*, *Lampropeltis*, and *Coluber*. It is not surprising that several species of crotalids were identified with a high prevalence of *O. ophiodiicola*, but it is noteworthy that several other crotalid species were not detected with *O. ophiodiicola* DNA at all. North American crotalids and *Nerodia* species have previously been shown to have a high prevalence of ophidiomycosis and may be uniquely sensitive to infection due to their environment (*Nerodia*) or morphology (pits in crotalids) (Allender et al., 2015c; McBride et al., 2015; Lorch et al., 2016). Recent studies in aquatic snakes in Kentucky have highlighted that aquatic snake susceptibility is under-represented in the literature and warrants careful investigation (McKenzie et al., 2018). It is possible that previous perceived susceptibility of crotalids represents a sampling bias, in which venomous species garner more attention. The lower observed prevalence in *Liodytes* and *Storeria* may be due to inherent resistance shared among closely related species, the smaller size of the snake resulting in smaller surface area for sampling and subsequent lower DNA quantity, or sharing life history traits or habitats that are less permissive to *Ophidiomyces* infection. Future investigations should characterize the mechanisms that lead to lower ophidiomycosis in these species because they may allow us to improve conservation efforts in other sensitive species.

Alternatively, the differences in prevalence may actually represent differences in susceptibility associated with habitat characteristics rather than taxonomic class. Therefore, the perceived higher rate of disease in Timber Rattlesnakes (Clark et al., 2013) and Eastern Massasaugas (Allender et al., 2011) might be due to the habitats that the populations of these species occupy. Future studies should focus on establishing the association between habitats used by snakes and *O. ophiodiicola* prevalence, specifically evaluating techniques for eDNA detection.

The distribution of ophidiomycosis has been known to extend across the eastern US (Allender et al., 2015; Lorch et al., 2016), but newer cases have been identified in the central and western US. The detection in Idaho represents the westernmost detection of this pathogen. It is unclear whether the pathogen is migrating to new habitats or being detected in previously untested sites. Identifying the reservoirs and modes of environmental transmission is integral to identifying intervention strategies that limit the impacts of the disease at both the individual and population levels.

Age class seems to play a significant role in the pathogenesis of ophidiomycosis as adults were more likely to be detected with *O. ophiodiicola* and apparent ophidiomycosis than juveniles.

This may be due to the fact that the pathogen is widespread in the environment (Allender et al., 2015c; McKenzie et al., 2018) and adults have an increased exposure to the pathogen over time. However, the impact of this pathogen on individual health remains unknown and the true impact on fitness needs to be evaluated. Specifically, monitoring body condition, mortality rate and reproductive output in affected snakes is critical to determining the conservation threat to snake species. For example, Pygmy Rattlesnakes in Florida with SFD have shown a decrease in body condition with increasing severity of infection (Lind et al., 2018). However, severity was not associated with recapture rate, and the authors observed severely affected snakes that were apparently able to clear infection (Lind et al., 2018). Conversely, Eastern Massasaugas in Michigan with ophidiomycosis were observed moving long distances less frequently and seeking basking sites more often late in the active season, indicating an energetic cost to infection compared to uninfected snakes in the same habitat (Tetzlaff et al., 2018).

Ophidiomycosis epidemiologic investigations have required a collaborative effort between biologists, veterinarians, and land managers and have produced a great deal of data about the distribution of this disease. However, it is not the only conservation threat to snakes, and may not even be the only disease facing species of conservation concern. At a time when wildlife diseases are increasingly more important for wildlife populations and public health, and wildlife serve as reservoirs for a wide variety of diseases, the need for early detection, or, ideally, prevention of the next disease event, has never been greater. Future health assessments, pathogen detection, and assessment of contaminant exposure in these snake populations may allow us to identify trends and new threats to both snakes and other wildlife species.

## **BENEFITS TO MILITARY**

The results of this investigation provide important baseline snake health information to participating DoD sites nationwide. Installation natural resource managers benefit from knowing whether or not ophidiomycosis or its causative agent, *O. ophiodiicola*, was detected in snakes inhabiting their landscape. Furthermore, this study provides critical large-scale insight on spatial and taxonomic variability needed to understand how best to minimize disease impact, particularly for imperiled species. Throughout the project participating installation personnel were provided technical training and education about ophidiomycosis natural history, identification, biosecurity measures, and sampling techniques through numerous email communications, electronic documents/protocols, and an in-person training session at the 2018 National Military Fish and Wildlife Association (NMFWA) conference. In addition to field and lab results, military natural resource personnel have a better understanding of what signs and symptoms to look for in snakes with ophidiomycosis, as well as how to conduct sampling for the disease.

This study represents an impetus for installations to justify wildlife management funding requests, proactively plan and prepare, and take mitigation action, where appropriate. Ultimately,

this effort benefits DoD by aiding in the prevention of restrictions to military readiness as a result of degrading ecosystem health. Results from this project apply not only directly to each participating installation as they assess the threat of ophidiomycosis at their property, but also to non-participating installations that may share similar habitat features, geographic proximity, or species presence. Continued communication and partnerships between military installations nationwide, as well as non-military conservation and wildlife stakeholders (e.g. US Fish and Wildlife, USGS, research universities) will be key to managing this disease moving forward.

## **MANAGEMENT IMPLICATIONS**

Ophidiomyces appears to be widespread throughout much of the eastern US and must now be considered a threat to snakes in the western US and Puerto Rico. The direct impact on individual snake health and subsequently biodiversity is unknown at this time. Clinical signs were present in snakes on DoD lands, so there is potential to cause either direct mortality or reduce overall fitness of snakes in these areas. Several imperiled species are susceptible to infection, most notably, at this time, the Indigo Snake. Future investigations should determine the impact this pathogen has on growth, reproduction, and survival, specifically in this species, and how landscape use affects prevalence. This study has provided information to each installation on the specific presence of this pathogen across their landscape, which should inform biosecurity measures. Several effective disinfectants have been published and should be used in conjunction with physical removal of dirt and debris to achieve appropriate biosecurity. Furthermore, the absence of this pathogen in several western bases signifies an opportunity to prevent introduction by implementing strict biosecurity measures to the extent practicable. Snakes serve an important role in the ecosystem and diseases such as ophidiomycosis may threaten their sustainability or require intervention that could result in limits on military training with increased protections.

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Table 1. Sample sizes and result of *Ophidiomyces* testing on military installations, by state, where snakes were sampled in 2018.

<b>State</b>	<b>Installation</b>	<b>Sample size</b>	<b>Detected with <i>Ophidiomyces</i></b>
<i>Alabama</i>			
	US Army Garrison Redstone	14	2
<i>California</i>			
	Marine Corps Base Camp Pendleton	24	0
	Naval Air Weapons Station China Lake	14	0
	Fort Hunter Liggett	5	0
	Parks Reserve Forces Training Area	5	0
	Beale AFB	4	0
	Edwards AFB	2	0
<i>Colorado</i>			
	USAF Academy	11	0
	Fort Carson/Pinon Canyon	10	0
<i>Florida</i>			
	Eglin Installation Complex	9	0
	Homestead AFB	4	0
	Hurlburt Field	3	1
<i>Georgia</i>			
	Fort Stewart	35	21
<i>Idaho</i>			
	Orchard Combat Training Center	48	1
<i>Indiana</i>			
	Naval Support Activity Crane	6	0
<i>Kansas</i>			
	Fort Riley	17	6
	Fort Leavenworth	15	1
	McConnell AFB	5	1
	Smoky Hill ANG Range	4	0
<i>Kentucky</i>			
	Fort Campbell	15	3
	Wendell H. Ford Regional Training Center	3	0
<i>Massachusetts</i>			
	Camp Edwards	17	5
<i>Maryland</i>			
	Naval Air Station Patuxent	35	6
	Blossom Point Research Facility	9	5
	Navy Support Facility Indian Head	6	1
	Adelphi Laboratory Center	5	2
<i>Maine</i>			
	Naval Survival, Evasion, Resistance and Escape School	5	0
	Naval Support Activity Cutler	2	0
	Great Pond Outdoor Adventure Center	1	0

<i>Michigan</i>			
	Camp Grayling	36	
<i>Minnesota</i>			
	Camp Ripley	12	0
	Arden Hills Army Training Site (AHATS)	3	0
<i>North Carolina</i>			
	Marine Corps Base Camp Lejeune	16	5
	Fort Bragg	7	0
<i>New Hampshire</i>			
	New Boston AFS	22	3
	Pembroke	13	1
<i>New Jersey</i>			
	Joint Base Maguire-Dix-Lakehurst	4	0
<i>New Mexico</i>			
	White Sands Missile Range	21	0
<i>Nevada</i>			
	Nellis AFB	17	0
<i>New York</i>			
	West Point Military Reservation	18	3
<i>Oklahoma</i>			
	Fort Sill	13	3
	Tinker AFB	7	0
<i>Pennsylvania</i>			
	Fort Indiantown Gap	20	8
<i>Puerto Rico</i>			
	Fort Buchanan	7	1
<i>South Carolina</i>			
	Parris Island	21	14
	Fort Jackson Army Installation	5	0
	Marine Corps Air Station Beaufort	3	3
<i>Tennessee</i>			
	Arnold Air Force Base	2	1
<i>Texas</i>			
	Joint Base San Antonio	4	0
<i>Utah</i>			
	Camp Williams	5	0
<i>Virginia</i>			
	Fort Lee	21	8
	Fort Eustis	6	0
<i>Washington</i>			
	Joint Base Lewis McChord Yakima	9	0
<i>Wisconsin</i>			
	Fort McCoy	22	6
<i>Wyoming</i>			
	Francis E. Warren AFB	10	0

Table 2. Sample sizes by species for snakes assayed for *Ophidiomyces ophiodiicola* on military installations from 2018.

<b>Common Name</b>	<b>Scientific Name</b>	<b>Sample Size</b>
<i>Florida Cottonmouth</i>	<i>Agkistrodon conanti</i>	4
<i>Eastern Copperhead</i>	<i>Agkistrodon contortrix</i>	13
<i>Broad-banded Copperhead</i>	<i>Agkistrodon laticinctus</i>	1
<i>Northern Cottonmouth</i>	<i>Agkistrodon piscivorus</i>	4
<i>Glossy Snake</i>	<i>Arizona elegans</i>	3
<i>Common Wormsnake</i>	<i>Carphophis amoenus</i>	15
<i>Puerto Rican Boa</i>	<i>Chilabothrus inornatus</i>	7
<i>Mohave Shovel-nosed Snake</i>	<i>Chionactis occipitalis</i>	3
<i>North American Racer</i>	<i>Coluber constrictor</i>	69
<i>Coachwhip</i>	<i>Coluber flagellum</i>	13
<i>Striped Whipsnake</i>	<i>Coluber taeniatus</i>	9
<i>Eastern Diamond-backed Rattlesnake</i>	<i>Crotalus adamanteus</i>	19
<i>Western Diamond-backed Rattlesnake</i>	<i>Crotalus atrox</i>	9
<i>Sidewinder</i>	<i>Crotalus cerastes</i>	3
<i>Timber Rattlesnake</i>	<i>Crotalus horridus</i>	3
<i>Western Rattlesnake</i>	<i>Crotalus oreganus</i>	33
<i>Red Diamond Rattlesnake</i>	<i>Crotalus ruber</i>	3
<i>Prairie Rattlesnake</i>	<i>Crotalus viridis</i>	2
<i>Ring-necked Snake</i>	<i>Diadophis punctatus</i>	15
<i>Eastern Indigo Snake</i>	<i>Drymarchon couperi</i>	19
<i>Red-bellied Mudsnake</i>	<i>Farancia abacura</i>	2
<i>Eastern Hog-nosed Snake</i>	<i>Heterodon platirhinos</i>	10
<i>Desert Nightsnake</i>	<i>Hypsiglena chlorophaea</i>	1
<i>California Kingsnake</i>	<i>Lampropeltis californiae</i>	6
<i>Prairie Kingsnake</i>	<i>Lampropeltis calligaster</i>	6
<i>Western Milksnake</i>	<i>Lampropeltis gentilis</i>	7
<i>Eastern Kingsnake</i>	<i>Lampropeltis getula</i>	2
<i>Eastern Milksnake</i>	<i>Lampropeltis triangulum</i>	16
<i>Three-lined Boa</i>	<i>Lichanura trivirgata</i>	2
<i>Glossy Swampsnake</i>	<i>Liodytes rigida</i>	1
<i>Plain-bellied Watersnake</i>	<i>Nerodia erythrogaster</i>	2
<i>Southern Watersnake</i>	<i>Nerodia fasciata</i>	4
<i>Diamond-backed Watersnake</i>	<i>Nerodia rhombifer</i>	1
<i>Common Watersnake</i>	<i>Nerodia sipedon</i>	27

<i>Rough Greensnake</i>	<i>Opheodrys aestivus</i>	9
<i>Smooth Greensnake</i>	<i>Opheodrys vernalis</i>	2
<i>Eastern Ratsnake</i>	<i>Pantherophis alleghaniensis</i>	33
<i>Great Plains Ratsnake</i>	<i>Pantherophis emoryi</i>	4
<i>Red Cornsnake</i>	<i>Pantherophis guttatus</i>	5
<i>Western Ratsnake</i>	<i>Pantherophis obsoletus</i>	8
<i>Western Foxsnake</i>	<i>Pantherophis ramspotti</i>	3
<i>Gray Ratsnake</i>	<i>Pantherophis spiloides</i>	20
<i>Spotted Leaf-nosed Snake</i>	<i>Phyllorhynchus decurtatus</i>	4
<i>Gophersnake</i>	<i>Pituophis catenifer</i>	65
<i>Eastern Pinesnake</i>	<i>Pituophis melanoleucus</i>	10
<i>Burmese Python</i>	<i>Python bivittatus</i>	1
<i>Long-nosed Snake</i>	<i>Rhinocheilus lecontei</i>	3
<i>Eastern Patch-nosed Snake</i>	<i>Salvadora grahamiae</i>	1
<i>Eastern Massasauga</i>	<i>Sistrurus catenatus</i>	38
<i>Western Massasauga</i>	<i>Sistrurus tergeminus</i>	4
<i>Western Groundsnake</i>	<i>Sonora semiannulata</i>	2
<i>Dekay's Brownsnake</i>	<i>Storeria dekayi</i>	9
<i>Red-bellied Snake</i>	<i>Storeria occipitomaculata</i>	2
<i>Terrestrial Gartersnake</i>	<i>Thamnophis elegans</i>	25
<i>Two-striped Gartersnake</i>	<i>Thamnophis hammondi</i>	1
<i>Eastern Ribbonsnake</i>	<i>Thamnophis saurita</i>	2
<i>Common Gartersnake</i>	<i>Thamnophis sirtalis</i>	70
<i>Smooth Earthsnake</i>	<i>Virginia valeriae</i>	2

Table 3. Free-ranging snake species detected with *Ophidiomyces ophiodiicola* for the first time during surveillance conducted on military installations, 2018.

<b>Common Name</b>	<b>Scientific Name</b>	<b>Location</b>
<i>Puerto Rican Boa</i>	<i>Chilabothrus inornatus</i>	<i>Puerto Rico</i>
<i>Great Plains Ratsnake</i>	<i>Pantherophis emoryi</i>	<i>Kansas</i>
<i>Western Milksnake</i>	<i>Lampropeltis gentilis</i>	<i>Kansas</i>
<i>Western Foxsnake</i>	<i>Pantherophis ramspotti</i>	<i>Wisconsin</i>

Table 4. Akaike Information Criteria (AIC) rankings of the top 5 logistic regression models predicting ophidiomycosis classification in species of snakes sampled on military installations in 2018. Sp = species, A = age class, S = state. K=number of parameters, AICc=AIC for small sample sizes, Delta AICc=change in AICc from the top model, AICc weight=proportion of variance in the data that the model explains (ideal =1.0), Cum. Wt = the cumulative weight of this model and all above it that explains the variance in the data, LL=log likelihood, the natural logarithm of the likelihood of the model (ideal values are lower).

Model	K	AICc	Delta AICc	AICcWt	Cum.Wt	LL
S+A	33	637.56	0	0.96	0.96	-283.78
S	31	644.62	7.06	0.03	0.99	-289.55
S+A+Sp	59	647.12	9.55	0.01	1	-257.94
S+Sp	57	649.04	11.48	0	1	-261.36
A+Sp	30	656.31	18.75	0	1	-296.51

Table 5. Odds ratios for states that were significantly associated with ophidiomycosis sampled in different states on military installations in 2018. CI = Confidence interval.

	Odds ratio	95% CI	p value
Georgia	5.28	1.31-21.51	0.027
Massachusetts	6.0	1.26-28.55	0.041
Pennsylvania	8.75	1.76-43.6	0.009
Virginia	3.64	0.91-14.61	0.033



Table 6. AIC rankings of the top 5 logistic regression models predicting ophidiomycosis classification detection in snake species sampled on military installations in 2018. Sp = species, A = age class, I = installation. K=number of parameters, AICc=AIC for small sample sizes, Delta AICc=change in AICc from the top model, AICc weight=proportion of variance in the data that the model explains (ideal =1.0), Cum. Wt = the cumulative weight of this model and all above it that explains the variance in the data, LL=log likelihood, the natural logarithm of the likelihood of the model (ideal values are lower).

Model	K	AICc	Delta AICc	AICcWt	Cum.Wt	LL
I + A	58	636.08	0	0.96	0.96	-253.65
I	56	642.53	6.45	0.04	1	-259.33
A + Sp	30	656.31	20.23	0	1	-296.51
I + A + Sp	83	660.5	24.42	0	1	-233.61
I + Sp	81	662.22	26.14	0	1	-237.16

Table 7. Significant predictors of ophidiomycosis classification in species of snakes sampled on military installations in 2018. A positive estimate is associated with an increased probability of ophidiomycosis.

	Estimate	Std. Error	z value	Pr
Intercept	0.7	1.5652	0.447	0.6547
Age <sub>Juvenile</sub>	-1.1552	0.369	-3.131	0.00174

Table 8. Prevalence of possible ophidiomycosis (clinical signs in the absence of *Ophidiomyces* qPCR positive result) in species of snakes sampled on military installations in 2018.

<b>Common Name</b>	<b>Scientific Name</b>	<b>N for Possible ophidiomycosis</b>	<b>Prevalence</b>
<i>Timber Rattlesnake</i>	<i>Crotalus horridus</i>	2	67%
<i>Eastern Ribbonsnake</i>	<i>Thamnophis saurita</i>	1	50%
<i>Puerto Rican Boa</i>	<i>Chilabothrus inornatus</i>	3	43%
<i>Common Wormsnake</i>	<i>Carphophis amoenus</i>	6	40%
<i>Eastern Pinesnake</i>	<i>Pituophis melanoleucus</i>	3	30%
<i>Western Milksnake</i>	<i>Lampropeltis gentilis</i>	2	29%
<i>Northern Cottonmouth</i>	<i>Agkistrodon piscivorus</i>	1	25%
<i>North American Racer</i>	<i>Coluber constrictor</i>	16	23%
<i>Striped Whipsnake</i>	<i>Coluber taeniatus</i>	2	22%
<i>Rough Greensnake</i>	<i>Opheodrys aestivus</i>	2	22%
<i>Ring-necked Snake</i>	<i>Diadophis punctatus</i>	3	20%
<i>Terrestrial Gartersnake</i>	<i>Thamnophis elegans</i>	4	16%
<i>Eastern Copperhead</i>	<i>Agkistrodon contortrix</i>	2	15%
<i>Coachwhip</i>	<i>Coluber flagellum</i>	2	15%
<i>Eastern Ratsnake</i>	<i>Pantherophis alleghaniensis</i>	5	15%
<i>Gray Ratsnake</i>	<i>Pantherophis spiloides</i>	3	15%
<i>Eastern Massasauga</i>	<i>Sistrurus catenatus</i>	5	13%
<i>Common Gartersnake</i>	<i>Thamnophis sirtalis</i>	9	13%
<i>Dekay's Brownsnake</i>	<i>Storeria dekayi</i>	1	11%
<i>Western Rattlesnake</i>	<i>Crotalus oreganus</i>	3	9%
<i>Common Watersnake</i>	<i>Nerodia sipedon</i>	2	7%
<i>Gophersnake</i>	<i>Pituophis catenifer</i>	4	6%
<i>Eastern Indigo Snake</i>	<i>Drymarchon couperi</i>	1	5%

Table 9. Prevalence percentages of ophidiomycosis classifications in species of snakes sampled on military installations in 2018.

Variable	Total n	Negative	<i>Ophidiomyces</i> present	Possible ophidiomycosis	Apparent ophidiomycosis
Age class					
Unknown	106	75.5%	6.6%	16.0%	1.9%
Juvenile	121	82.6%	9.9%	6.6%	0.8%
Adult	430	65.6%	10.5%	13.3%	10.7%
Lesions					
No	526	87.8%	12.2%	0%	0%
Yes	49	0%	0%	62.6%	37.4%

Supplemental Table 1. List of GenBank numbers for gene sequences of the cytochrome b gene in snakes sampled in 2018 at DoD military installations for ophidiomycosis. Sequences were used to generate a phylogenetic tree adapted from Figueroa et al. 2016.

Common Name	Scientific Name	GenBank Accession
<i>Eastern Copperhead</i>	<i>Agkistrodon contortrix</i>	EU483383.1
<i>Broad-banded Copperhead</i>	<i>Agkistrodon laticinctus</i>	EU483383.1
<i>Northern Cottonmouth</i>	<i>Agkistrodon piscivorus</i>	EU483436.1
<i>Florida Cottonmouth</i>	<i>Agkistrodon conanti</i>	EU483436.1
<i>Glossy Snake</i>	<i>Arizona elegans</i>	DQ902101.1
<i>Common Wormsnake</i>	<i>Carpophis amoenus</i>	AF471067.1
<i>Puerto Rican Boa</i>	<i>Chilabothrus inornatus</i>	KC329929.1
<i>Mohave Shovel-nosed Snake</i>	<i>Chionactis occipitalis</i>	GQ895857.1
<i>North American Racer</i>	<i>Coluber constrictor</i>	AY486914.1
<i>Coachwhip</i>	<i>Coluber flagellum</i>	AY486927.1
<i>Striped Whipsnake</i>	<i>Coluber taeniatus</i>	KP765660.1
<i>Eastern Diamond-backed Rattlesnake</i>	<i>Crotalus adamanteus</i>	JN620809.1
<i>Western Diamond-backed Rattlesnake</i>	<i>Crotalus atrox</i>	JN620811.1
<i>Sidewinder</i>	<i>Crotalus cerastes</i>	JN620812.1
<i>Timber Rattlesnake</i>	<i>Crotalus horridus</i>	AF337057.1
<i>Western Rattlesnake</i>	<i>Crotalus oreganus</i>	AF147871.1
<i>Red Diamond Rattlesnake</i>	<i>Crotalus ruber</i>	KP765661.1
<i>Prairie Rattlesnake</i>	<i>Crotalus viridis</i>	AF471066.1
<i>Ring-necked Snake</i>	<i>Diadophis punctatus</i>	AF471094.1
<i>Eastern Indigo Snake</i>	<i>Drymarchon couperi</i>	KP765662.1
<i>Red-bellied Mudsnake</i>	<i>Farancia abacura</i>	U69832.1
<i>Eastern Hog-nosed Snake</i>	<i>Heterodon platirhinos</i>	GU112412.1
<i>Desert Nightsnake</i>	<i>Hypsiglena chlorophaea</i>	KJ486459.1
<i>California Kingsnake</i>	<i>Lampropeltis californiae</i>	AF337058.1
<i>Prairie Kingsnake</i>	<i>Lampropeltis calligaster</i>	KF216289.1
<i>Eastern Kingsnake</i>	<i>Lampropeltis getula</i>	AF337071.1
<i>Eastern Milksnake</i>	<i>Lampropeltis triangulum</i>	KF216244.1
<i>Western Milksnake</i>	<i>Lampropeltis gentilis</i>	KF216224.1
<i>Three-lined Boa</i>	<i>Lichanura trivirgata</i>	U69844.1
<i>Glossy Swampsnake</i>	<i>Liodytes rigida</i>	AF471052.1
<i>Plain-bellied Watersnake</i>	<i>Nerodia erythrogaster</i>	AF402912.1
<i>Southern Watersnake</i>	<i>Nerodia fasciata</i>	AF402910.1
<i>Diamond-backed Watersnake</i>	<i>Nerodia rhombifer</i>	AF402915.1
<i>Common Watersnake</i>	<i>Nerodia sipedon</i>	AF402913.1

<i>Rough Greensnake</i>	<i>Opheodrys aestivus</i>	AF471057.1
<i>Smooth Greensnake</i>	<i>Opheodrys vernalis</i>	GQ927322.1
<i>Eastern Ratsnake</i>	<i>Pantherophis alleghaniensis</i>	AF283644.1
<i>Great Plains Ratsnake</i>	<i>Pantherophis emoryi</i>	AF337173.1
<i>Red Cornsnake</i>	<i>Pantherophis guttatus</i>	AM236349.1
<i>Western Ratsnake</i>	<i>Pantherophis obsoletus</i>	DQ538339.1
<i>Western Foxsnake</i>	<i>Pantherophis ramspotti</i>	FJ267684.1
<i>Gray Ratsnake</i>	<i>Pantherophis spiloides</i>	AF283643.1
<i>Spotted Leaf-nosed Snake</i>	<i>Phyllorhynchus decurtatus</i>	AF471083.1
<i>Gophersnake</i>	<i>Pituophis catenifer</i>	AF337112.1
<i>Eastern Pinesnake</i>	<i>Pituophis melanoleucus</i>	AF337100.1
<i>Burmese Python</i>	<i>Python bivittatus</i>	JX401131.1
<i>Long-nosed Snake</i>	<i>Rhinocheilus lecontei</i>	AF337109.1
<i>Eastern Patch-nosed Snake</i>	<i>Salvadora grahamiae</i>	KP765667.1
<i>Western Massasauga</i>	<i>Sistrurus tergeminus</i>	AY223610.1
<i>Western Groundsnake</i>	<i>Sonora semiannulata</i>	AF471048.1
<i>Dekay's Brownsnake</i>	<i>Storeria dekayi</i>	AF471050.1
<i>Red-bellied Snake</i>	<i>Storeria occipitomaculata</i>	AF402921.1
<i>Terrestrial Gartersnake</i>	<i>Thamnophis elegans</i>	EF417411.1
<i>Two-striped Gartersnake</i>	<i>Thamnophis hammondi</i>	AF420139.1
<i>Eastern Ribbonsnake</i>	<i>Thamnophis saurita</i>	AF420177.1
<i>Common Gartersnake</i>	<i>Thamnophis sirtalis</i>	AF402930.1
<i>Smooth Earthsnake</i>	<i>Virginia valeriae</i>	KF258656.1

Figure 1. Spatial distribution of *Ophidiomyces ophiodiicola* detection in snakes on military installations sampled in 2018. White = states not sampled, light grey = states with no detection of *O. ophiodiicola*, dark grey = states detected with *O. ophiodiicola*. White asterisks indicates a state/territory identified with *O. ophiodiicola* the first time in this study.

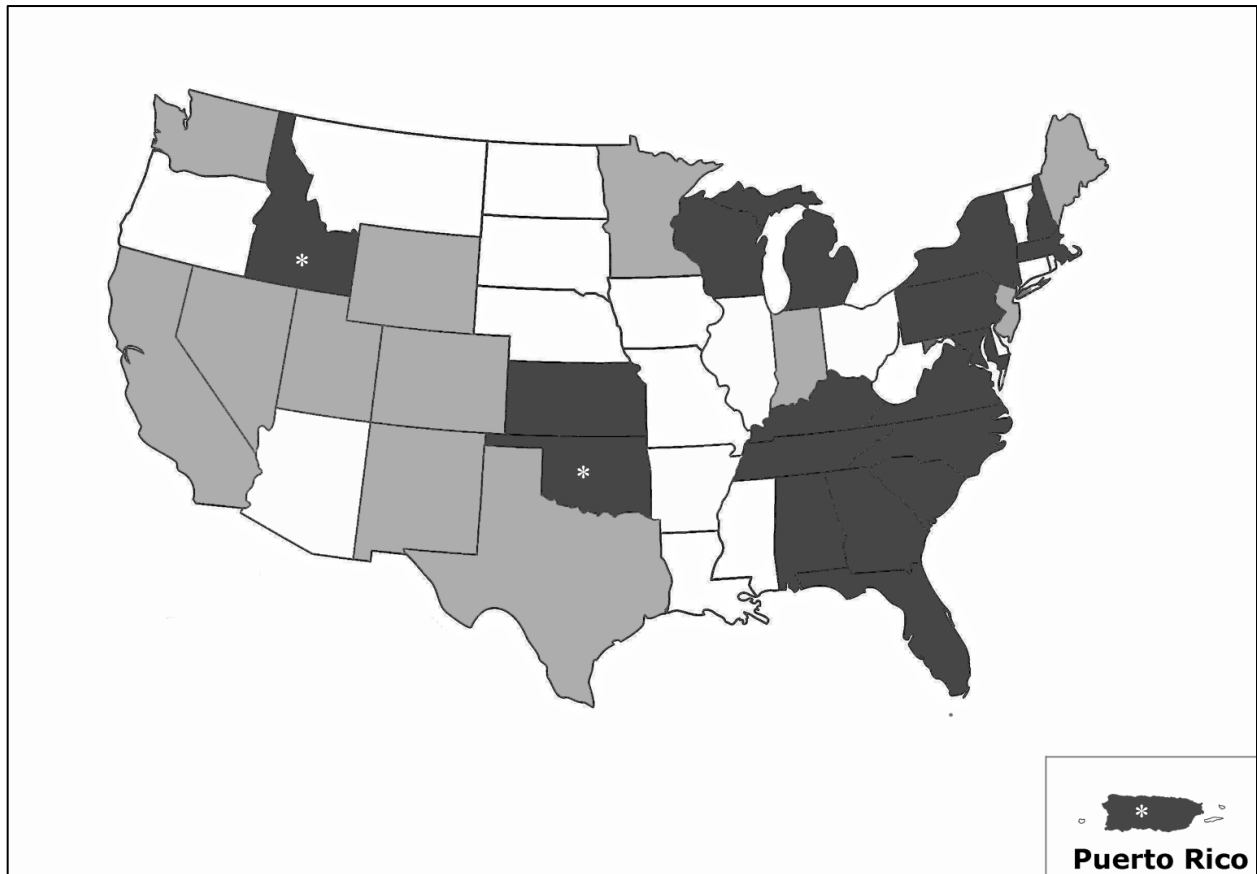


Figure 2. Spatial distribution and prevalence of ophidiomycosis in snakes on military installations sampled in 2018. Negative = no clinical signs and no qPCR detection of *Ophidiomyces ophiodiicola*; Present = qPCR positive AND no clinical signs; Possible = clinical signs present AND no qPCR detection; Apparent = qPCR detection AND clinical signs.

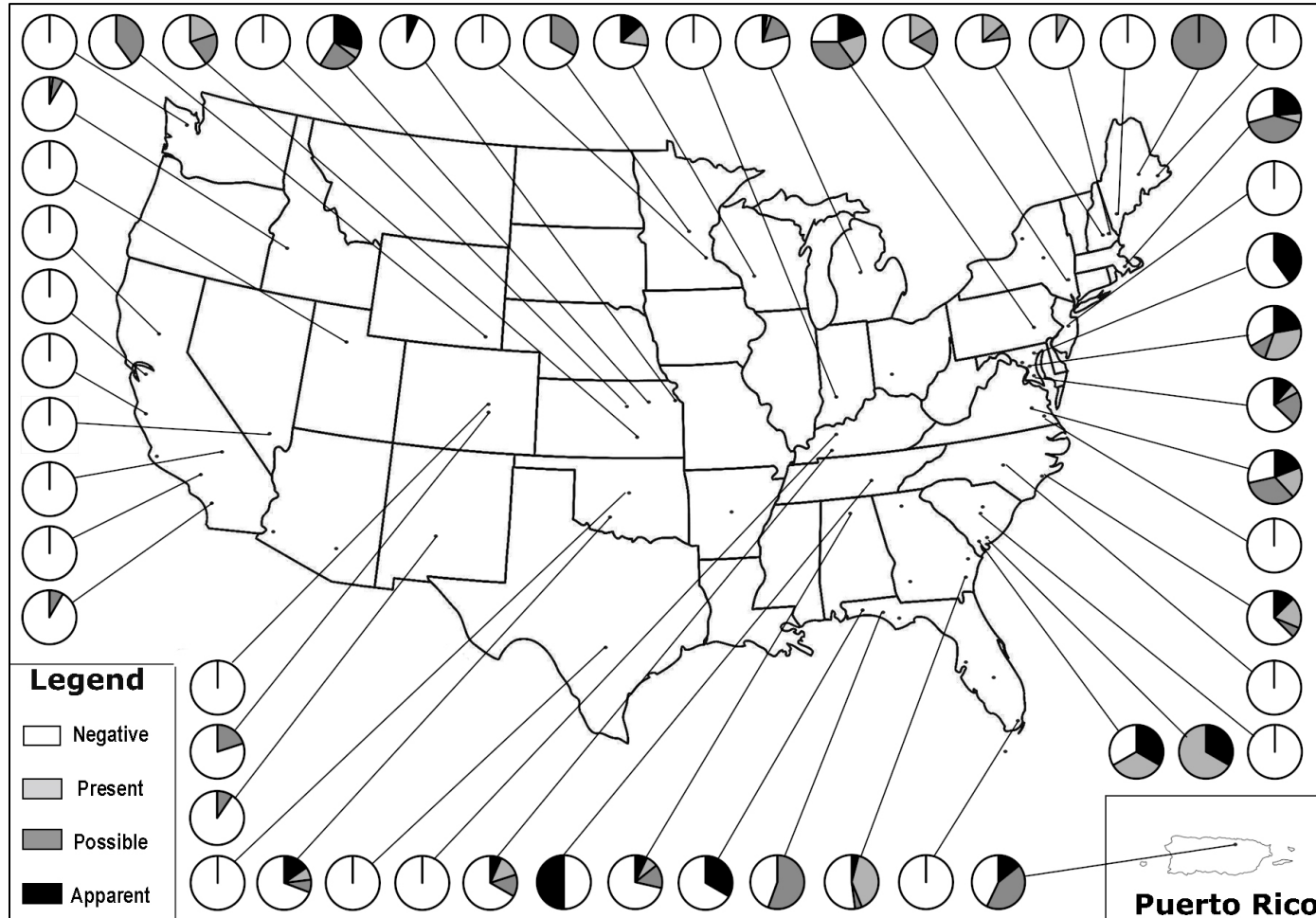


Figure 3. Phylogenetic analysis of snake species detected with *Ophidiomyces ophiodiicola* using qPCR from sampling on military installations in 2018. Green = 0% prevalence; yellow = 0-9.9%; light orange = 10-24.9%; dark orange = 25-49.9%; red = >50%.

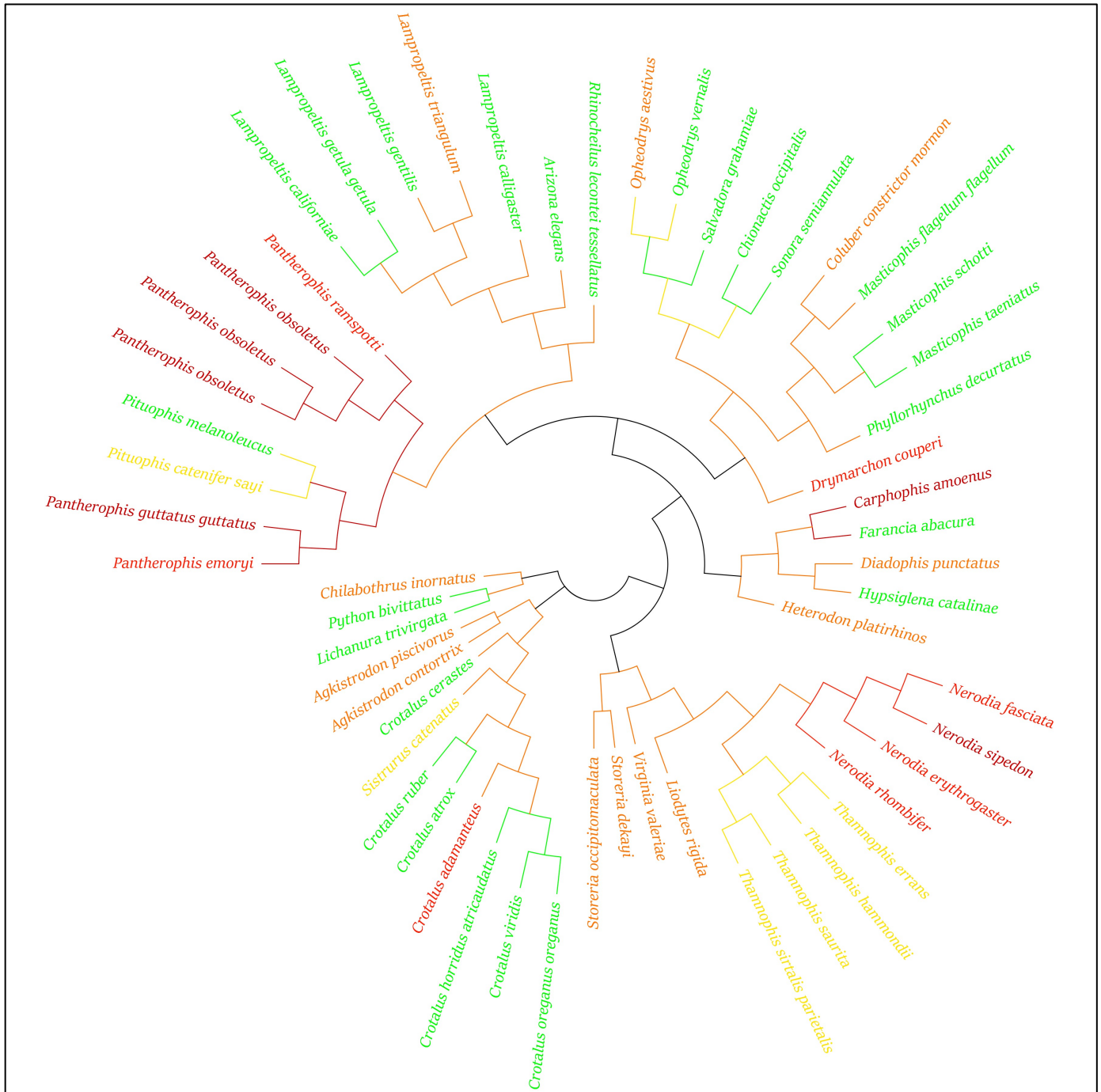




Figure 4. Photographs of swab sampling (A,B) of snakes for detection of *Ophidiomyces* on military installations in 2018. A ophidiomycosis (Snake fungal disease) lesion in *Crotalus oreganus helleri* (C), *Pituophis melanoleucus* (D), *Chilabothrus inornatus* (E), and *Pantherophis spiloides* (F).



Appendix 1. Snake sampling protocol sent to each participating Department of Defense installation.

### **DoD Snake Fungal Disease Sampling Protocol**

Thank you for participating in the DoD PARC Snake Fungal Disease (SFD) survey!

To get started, an instructional video specifically developed for this project can be found online at: <https://www.youtube.com/watch?v=PxuPCMppeIY&feature=youtu.be>. In addition, this protocol recaps the information in the video and can be brought in the field with you as a reference.

The sample kit you received in the mail includes the following items:

- ✓ Styrofoam shipping container in cardboard box
- ✓ 1 cold pack for return shipping
- ✓ 1 Ziploc bag
- ✓ 1 pre-paid shipping label for sending samples to the Wildlife Epidemiology Lab
- ✓ 1 UN 3373 Biological Substance Category B label for return shipping
- ✓ 1 Exempt Animal Specimen sticker for return shipping
- ✓ 3 printed copies of the data sheet
- ✓ 1 package of cotton tipped swabs
- ✓ 1 box containing 50 2mL Eppendorf tubes
- ✓ 1 ruler
- ✓ 1 permanent marker

### **Snake Handling:**

Be aware of potential venomous species on your installation and verify a snake is non-venomous before handling. Venomous snakes should not be captured for testing unless you have been specifically trained in your installation's protocol and have access to the proper equipment.

For basic information on snake safety and handling visit:

<https://www.youtube.com/watch?v=YMAN0HxHGCU>

Always wear disposable gloves when handling snakes. Snakes should be restrained firmly but gently and the head should be controlled by holding it at the base of the head to prevent bites. As much as possible, the snake's entire body should be supported.

### **Biosecurity Precautions:**

Biosecurity is critical to prevent transmission of snake fungal disease among snakes. Disposable gloves should be worn when handling any snake and should be changed between snakes. Any equipment that contacts a snake or the soil, such as boots, hooks, or buckets, should be cleaned with a solution of 10% bleach between snakes and/or between study areas. Organic matter such as mud or leaves should be removed before bleaching to maximize the efficacy of the disinfection. If multiple snakes are collected at once for later sampling, each should be placed separately in a clean bucket or pillowcase that is disinfected between animals.

### **Swabbing Procedure:**

#### **1. Physical exam:**

- a. Weigh the snake using a spring scale or in a clean container on a digital scale (if available)
- b. Measure snout-vent length (SVL) if possible
- c. Examine the snake for SFD lesions, making sure to fully observe the head, neck, back, belly, sides, and tail for any raised or discolored scales, pustules, crusts, or ulcers

- d. Record all findings on the data sheet including species, SVL, weight, lesion size (using ruler included) and location. Each lesion will be assigned a letter, as described on the data sheet, and lesions should be marked on the images of the snake on the data sheet
  - e. Take a “bird’s eye” photo of the entire snake with a ruler for scale; also photograph each lesion noted on the data sheet. In each photo, include a label with the installation code, snake number, and lesion letter. Include a ruler in the photos to indicate lesion size when possible
- 2. Swab collection:**
- a. Use the provided cotton tipped applicators to collect samples
  - b. Body samples (all snakes):
    - i. Pass a clean swab up and down the snake’s body 8 times, pressing firmly but gently with the side of the swab and turning the swab as you go. Make sure to sample the sides and dorsal body of the snake
    - ii. Quantity: For snakes without lesions just take the 2 whole body samples (8 passes with each swab, 16 passes total)
  - c. Lesion samples (snakes with lesions):
    - i. Use a separate clean cotton tipped applicator for each lesion and only swab the site of the lesion. Use pressure when swabbing. If any scales or scabs are removed during swabbing, include these with the swab in the provided Eppendorf tube
    - ii. Quantity: For snakes with lesions, take the two body samples mentioned above (8 passes each swab, 16 passes total) plus a firm swab from each lesion (up to 4 lesions)
  - d. Each swab should be placed in a clean, separate Eppendorf tube. Do not allow the swab to contact any other surfaces, animals, or swabs before storage. Place the swab in the tube and break off the excess swab handle before securely closing the lid of the tube
  - e. Label the tube with the date of collection, installation code, snake number, and swab letter: “a” or “b” for the body swabs and “c” and onward for any lesion swabs. An individual lesion should have the same letter on the data sheet, the label in the photo, and on the tube
3. After collecting all the necessary information and swabs from the snake, return the snake to the wild at the location where it was found

**Swab Storage and Shipping:**

Store the tubes in the provided cardboard box in the freezer until they are shipped to the Wildlife Epidemiology Lab using the provided shipping label. Sample boxes should be shipped in the provided Styrofoam cooler with the frozen icepack via overnight shipping. Data sheets should be included with the shipment and should be placed in a sealed plastic bag for protection.

Make sure to place the “UN 3373 Biological Substance Category B” and “Exempt Animal Specimen” stickers on the outside of the box before shipping.

Ship to:                   Dr. Matt Allender  
Wildlife Epidemiology Laboratory  
1729 VMBSB  
2001 S. Lincoln Ave.  
Urbana, IL 61802

**Questions or Concerns:**

Please contact Dr. Matt Allender ([mcallend@illinois.edu](mailto:mcallend@illinois.edu)) or Dr. Ellen Haynes ([haynes11@illinois.edu](mailto:haynes11@illinois.edu))

Appendix 2. Snake sampling data sheet sent to each participating Department of Defense installation.



### Snake Fungal Disease Surveillance



Page one; body swabs, all snakes

Date \_\_\_\_\_ Time \_\_\_\_\_ Observer \_\_\_\_\_  
 Installation \_\_\_\_\_ State \_\_\_\_\_ County \_\_\_\_\_  
 Latitude \_\_\_\_\_ Longitude \_\_\_\_\_  
 Habitat \_\_\_\_\_  
 Species \_\_\_\_\_ Snake ID \_\_\_\_\_  
 Sex \_\_\_ Age Class \_\_\_\_\_ SVL (cm) \_\_\_\_\_ Mass (g) \_\_\_\_\_  
 Behavior at capture: coiled \_\_\_ or stretched out \_\_\_ moving \_\_\_ or stationary \_\_\_  
 Alert and active when handled? yes \_\_\_ no \_\_\_  
 Skin Lesions: yes \_\_\_ no \_\_\_ **If yes, fill out page 2 of this form.**

**Instructions:** See swabbing protocol sheet and video for detailed instructions. Each snake encountered gets two separate swabs passed along the entire body eight times each (8 passes per swab, 16 passes total). Each body swab sample is placed in a separate tube. Your kit contains tubes labeled for your first few snakes. Use this labeling convention for the rest of your samples. Snake ID is an installation code assigned by us (01, 02, 03, etc) followed by 1, 2, 3, etc. for each snake. First body sample is then given “a” and second “b”. For example, the first body sample from the first snake from installation 01 would be: 01-1a and second body swab would be 01-1b.

**If lesions are present, see page two.**

Alert and active means a normal reaction of a snake to being handled, i.e. attempts to escape or even bite. Answer “no” to this section if the snake is limp or does not behave as above.

SVL is snout to vent length. Measure SVL if possible.

Wildlife Epidemiology Lab, 2001 S. Lincoln Ave., Urbana, IL [vetmed.illinois.edu/wel](http://vetmed.illinois.edu/wel)

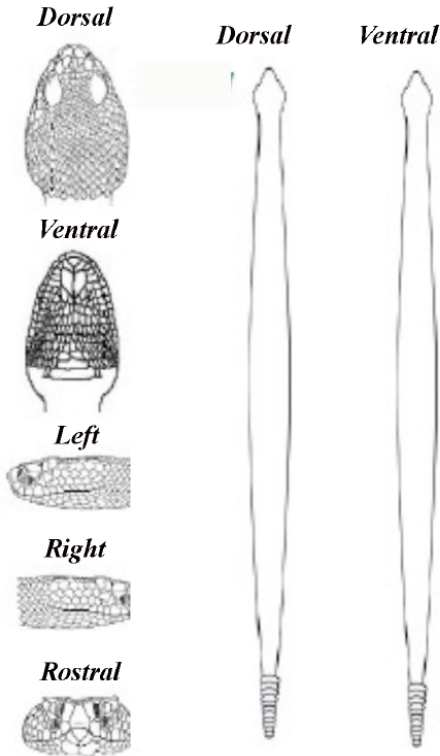


## Snake Fungal Disease Surveillance



Page two; lesion swabs, one form per snake

Species	Snake ID	
Lesion #	Lesion size (mm)	Lesion Location (head, dorsal body, ventral body, tail)
c		
d		
e		
f		



**Instructions:** See swabbing protocol sheet and video for additional details. Mark the location of each lesion on the diagram using lesion letter from table above. Measure lesion diameter with ruler. Each lesion swab is placed in a new tube. Label lesion swab tubes with same Snake ID from page 1, followed by c, d, e, etc. For example, the first lesion from Snake 01-1, would be labeled 01-1-c (because “a” and “b” were used for the first two body swabs). Only collect up to four lesion samples per snake.