**Department of Defense**
**Legacy Resource Management Program**

PROJECT NUMBER NR 21-005

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

Matthew C. Allender,[1,2] Amber Simmons,[1] Laura Adamovicz,[1] Albert Yu,[3] Christopher E. Petersen,[4] and Robert Lovich[5]

[1] Wildlife Epidemiology Laboratory, Veterinary Diagnostic Lab, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL; [2] Chicago Zoological Society, Brookfield, IL 60513; [3]Departement of Statistics, University of Illinois at Urbana-Champaign, Urbana, IL; [4] Naval Facilities Engineering Systems Command Atlantic, 6506 Hampton Blvd. Norfolk, VA 23508; [5]Naval Facilities Engineering Systems Command Southwest, 1220 Pacific Highway, San Diego, CA 92132

August 22, 2023

# TABLE OF CONTENTS

# 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** |
| **NMFWA** | **National Military Fish and Wildlife Association** |
| **AIC** | **Akaike Information Criteria** |

# ABSTRACT

Ophidiomycosis (formerly referred to as Snake Fungal Disease, SFD), a pathogen on the North American landscape caused by the fungal pathogen *Ophidiomyces ophidiicola*, poses a threat to snake population health and stability directly through mortality and indirectly through illness-associated reductions in reproductive output or physiologic dysfunction. It has been documented in over 20 genera of wild individuals and snakes under human care. The study was to expand the initial 657-snake survey from 2018-2019 that detected ophidiomycosis in 17% of snakes on DoD lands. It also identified the first reports of this pathogen in Oklahoma, Idaho, and Puerto Rico. During 2021-2022, 81 snake species on 48 military installations within 32 states were evaluated, representing over 1900 swabs from 826 individuals. One hundred and fifty-eight (19.2%) individuals from 36 species were detected with *O. ophidiicola* DNA, including the first reports of the pathogen in free-ranging snakes in New Mexico. Apparent ophidiomycosis (lesions and *O. ophidiicola* qPCR positive) was observed in 84 individuals, *O. ophidiicola* qPCR positive was detected in 74 individuals in the absence of clinical signs, 72 snakes had possible ophidiomycosis (lesions but *O. ophidiicola* qPCR negative), and 596 were qPCR negative and lacked lesions. Results conclude that *Ophidiomyces* 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.

Snakes inhabit the ecosystems of Department of Defense (DoD) lands across the globe, and conservation efforts aimed at minimizing threats to snakes are implemented as part of the overall management of natural resources on military lands. Therefore, understanding potential threats to these species is of conservation interest and may have implications for military training activities, in which federal natural resource management regulations play a role. This survey also adds to the growing literature that demonstrates the widespread nature of this pathogen.

# INTRODUCTION

Wildlife health is increasingly identified as a key component of conservation programs, and disease has been associated with population threats of several species, including mammals (bats with White Nose Syndrome), birds (avian influenza multi-species outbreaks), amphibians (chytridiomycosis), and reptiles (ophidiomycosis). Integrating health assessment and pathogen surveys are necessary to develop baseline population prevalences, identify emerging pathogens, and monitor response to habitat alteration or improvements.

Ophidiomycosis is a disease primarily affecting snakes caused by the fungi *Ophidiomyces ophidiicola*. This pathogen was reported in North America in the early 2000s, but its distribution has since been expanded both temporally and spatially. The newest reports identify positive snakes into the early 1900s using museum specimens in North America (Lorch et al., 2021) and late 1950s in Europe (Origgi et al., 2022). Additionally, affected snakes have now been identified in Europe and Asia (Takami et al., 2021). Phylogenetic analysis has demonstrated that isolates from snakes in North America have been introduced multiple times within the last 100 years, while isolates collected from snakes between Europe and North America likely diverged 2000 years ago (Ladner et al., 2022). Surveillance efforts of free-ranging North America snakes identified several subclades and recombinant clades occurring within the same geographic area and species (Haynes, 2021) suggesting that *Ophidiomyces* diversity has a more historical occurrence rather than point source introduction. This is supported by recent meta-analysis suggesting that *Ophidiomyces* is a common pathogen on the landscape, but historical under-surveillance led to its classification as emerging rather than being a true endemic disease (Davy et al., 2021).

Natural resources on military lands support a large percentage of endangered habitats and species in the US (Stein et al, 2008; Aycrigg et al, 2015), including two-thirds of all amphibian and reptile species documented in the continental US (Petersen et al., 2018). The DoD manages herpetofauna under guidance from a comprehensive strategic plan (Lovich et al., 2015) and implements an overall ecosystem management approach to maintain and/or restore healthy land and water habitats in support of military training (Benton et al., 2008). Military lands are home to 131 snake species, several either currently listed or candidates for listing as threatened or endangered by the USFWS (e.g. Eastern Indigo Snake, Louisiana Pinesnake, Black Pinesnake, Brown Gartersnake, Eastern Massasauga) (Aycrigg et al, 2015). Therefore, understanding potential threats to these species is of conservation interest and may have implications for military training activities, in which federal natural resource management regulations play a role.

Investigating pathogen occurrence in reptiles on DoD lands aids in conservation missions, but to date, only a single large-scale effort has been made to investigate the health of snakes, which was Phase 1 of this project (Allender et al., 2020). Ophidiomycosis (formerly known as Snake Fungal Disease; SFD) is an infectious disease of free-ranging and managed snakes (Lorch et al., 2016; Sigler et al., 2013), caused by the fungus *Ophidiomyces ophidiicola* (Allender et al., 2015; Lorch et al., 2015). The pathogen has been observed in more than 30 species of snakes in the US, Europe, and Asia (Lorch et al., 2016; Allender et al., 2015; Burbrink

et al 2017; Franklinos et al., 2017; Davy et al., 2021; Haynes, 2021; Takami et al., 2021). Clinical signs of SFD include accelerated shed cycles, displaced and/or discolored scales, crusting, granulomas, nodules, and swelling or disfiguration of infected tissues (Baker et al., 2019).

# PROJECT DESCRIPTION

Global landscapes have experienced unprecedented changes, largely due to anthropogenic activities, and many habitats no longer resemble the ecosystems in which species evolved. These landscape changes are associated with population and species declines via several factors, including habitat destruction, climate change, and infectious disease. Deteriorating ecosystem health further threatens the sustainability of natural resource management, the prevention of human disease, and the success of efforts to conserve biodiversity.

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 including viruses such as hantavirus and bacteria such as Lyme disease. Furthermore, healthy snake populations promote overall biodiversity, which is integral for maximizing ecosystem health and productivity. Three hundred and nineteen snake species world-wide are at risk due to global landscape changes, representing 15% of known species with a population assessment.

This project specifically set out to: 1. Determine the occurrence of ophidiomycosis on DoD lands across the US and its territories using qPCR in combination with clinical signs, and 2. Determine the significant demographic, spatial, or taxonomic associations with ophidiomycosis. These data will provide important health data for snakes on DoD sites.

# MATERIALS AND METHODS

**STUDY SITES–** *O. ophidiicola* sampling kits were provided to 68 military installations, and samples were received from 43 (Table 1). The sites ranged across the US and a number of habitat types.

**FIELD SAMPLE COLLECTION–** Snake surveys for *O. ophidiicola* began on 5 April 2020 and samples were collected through 18 October 2022 (30 months). Upon capture, all snakes were visually inspected for skin lesions, scabs, or other areas that may indicate an ophidiomycosis infection.

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 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 five 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.

**QUANTITATIVE PCR–**DNA extraction and 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 300U 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.

**OPHIDIOMYCES CATEGORIES**— 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. ophidiicola* 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).

**STATISTICAL ANALYSIS–**Prevalence of each ophidiomycosis category was estimated by calculating the 95% binomial confidence interval in total and modeled by sex, age class, installation, and month. Variables 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). To increase the predictive power of the models, species with less than 5 individuals represented were removed. After removing species with fewer than 5 individuals represented, a series of ordinal cumulative logistic regressions were fit in R using polr() in the MASS package for ophidiomycosis status (four output categories). A combination of Species, Installation Name, State, Age Class, and Sex were considered. For these models, species were not able to be used as a predictor for these models due to high standard errors attributed to species with insufficient observations in some ophidiomycosis categories.

Due to the high overlap between installation and state, these two variables were never used in the same model. Logistic regression was performed using *Ophidiomyces* detection as the response. Next, an information theoretic approach was used to determine which model from the candidate set performed best using the model with the lowest AIC. All factors were included. Interaction terms were not pursued due to sample size constraints. To analyze what snakes are more susceptible to becoming assigned into the apparent ophidiomycosis category given that they have tested positive for the disease, we created a subset of the data for only observations that tested positive. Positive and negative predictive values were calculated from 2x2 tables to evaluate the usefulness of using skin lesions for detection of *Ophidiomyces*. Statistical significance was assessed at $\alpha = 0.05$ and all statistical analyses were conducted using commercial software (R Development Core Team, 2023)

# RESULTS

**GENERAL SURVEY RESULTS–** Over 5,700 qPCR reactions for 1916 swabs were assayed from 826 individual snakes. Snakes were sampled from 48 military installations from 32 states (Table 1). Snakes sampled represented 81 species (Table 2). There were 554 adults, 145 juveniles, and 127 snakes of unknown age sampled.

**OPHIDIOMYCES DETECTION AND OPHIDIOMYCOSIS CLASSIFICATION–** Individuals were captured once each and swabbed from 1 to 7 times. Skin lesions were observed in 156 individuals for an overall prevalence of 18.9%. *Ophidiomyces ophidiicola* DNA was detected in samples from 158 snakes for a prevalence of 19.2% (95% CI: 16.5 – 22.0%). Eighteen states/territories were detected with *O. ophidiicola* DNA, including for the first time New Mexico. All four categories of ophidiomycosis were represented in this study. Most animals were ophidiomycosis negative (n=596), with *Ophidiomyces* present (n=74), possible ophidiomycosis (n=72), and apparent ophidiomycosis (n=84) occurring less frequently. Prevalence at each base ranged from 0% to 100%.

**LOGISTIC REGRESSION MODELS–** The final data set for the model included 751 individuals from 32 species representing 509 adults, 131 juveniles, and 111 snakes of unknown age. There were 117 female, 162 male, and 412 snakes with unknown sex included. The final data set included 543 negative, 68 *Ophidiomyces* present, 64 possible ophidiomycosis, and 76 apparent ophidiomycosis individuals.

For the ordinal regression, installation was a much better predictor than state, as a model using only installation reduced the residual deviance to 980.8 (null deviance = 1342.229, AIC = 1102.8) versus a residual deviance of 1064.9 and AIC = 1132.9 for the state-only model. The final cumulative ordinal model used installation and age class as predictors. This model had a residual deviance of 975.303 and AIC of 1101.3. Sex was not a good predictor, as the predictor

failed to be significant, only lowered the residual deviance to 972, and increased the AIC. These results show that while the installation location is the best predictor to explain variability, even the full model is unable to explain most of the variability. A juvenile age is associated with -0.429 log odds to move up in ophidiomycosis categories. Though the differences between juveniles and adults were not statistically significant (p value = 0.129), the cumulative ordinal model suggests that age class does play some role in explaining ophidiomycosis status, even after controlling for location data.

According to AIC and residual deviance, the best binary logistic regression model was the same as in ordinal regression: using installation and age. Residual deviance = 432.9, Null Deviance = 734.1, AIC = 554.9. Age class was not significant in this model. This model detected five installations with a significantly greater odds of detection ($p < 0.05$): Fort Lee ($p=0.008$), MCB Camp Lejeune ($p=0.009$), Naval Weapons Station Yorktown ($p=0.027$), and PA Department of Military and Veterans Affairs ($p=0.008$). Using a model with species + age is worse since it gives a higher residual deviance (543.9) and AIC (615.9). When modeled with species, the juvenile age class has a significant result at alpha = 0.05 and is 0.35 times as likely to have a positive result compared to adults (logit=-0.64, p = 0.049). In this particular analysis, only *Nerodia sipedon* (Northern Water Snake) was identified as having a significant change in log odds (logit = 2.83, p = 0.0147). However, *Carphophis amoenus* (Eastern Worm Snake), *Coluber constrictor* (Eastern Racer), *Crotalus horridus* (Timber Rattlesnake), *Lampropeltis elapsoides* (Scarlet Kingsnake) were also identified as having higher log odds of ophidiomycosis detection with marginal significance. *Drymarchon couperi* (Indigo Snake) was shown in the data to have a high risk, but did not appear significant in the analysis due to an extremely high standard error (there were 15 samples of *Drymarchon couperi*, and all 15 tested positive). A number of snakes had low risk but were not flagged as significant due to receiving no positive samples.

Next, a logistic regression was fit using apparent status as the response and species as the predictor (n=146) to determine species susceptibility. The resulting model gave a residual deviance of 162.0 on 162 degrees of freedom with a null deviance of 202.4 with df=121. Three species were found to be significant. *Coluber constrictor* (logit = .938, p = 0.017) and *Drymarchon couperi* (logit = 1.386, p = 0.032) were more likely to exhibit symptoms than other species while ophidiomycosis was present. While *Nerodia sipedon* was found in the previous model to be more likely to contract the disease, it is significantly less likely to exhibit signs (logit = -1.179, p= 0.039).

# DISCUSSION

This project set out to expand surveillance of snakes on military lands that the first surveillance effort initiated in 2018-2019. The overall prevalence was similar (n =668; 19.2% compared to previous finding of 17%) indicating this pathogen remains on the landscape on military

installations nationwide. Ophidiomycosis has potentially serious consequences for the success of snake conservation efforts in North America (Allender et al., 2015c; Lorch et al., 2016; Ladner et al. 2022). *O. ophidiicola* DNA was detected in 36 species or subspecies, all of which have previously been identified in the literature. Species identified with *Ophidiomyces* are from various taxa, locations, and age classes and contain common and rare species. This survey adds to the growing literature that demonstrates the widespread nature of this pathogen.

High prevalence of ophidiomycosis in certain species was consistent with previous findings (*Drymarchon* [Indigo snake], *Coluber* [Racers]), while other species were also identified in this surveillance effort (*Nerodia* [watersnakes], *Lampropeltis* [kingsnakes], *Carphophis* [worm snakes]). It is interesting to note that logistic regression models only identified *Nerodia sipedon* (Northern Water Snake) has having the highest odds of being detected, but this likely represents a statistical significance and not truly epidemiological significance. *Nerodia* in previous surveys on military lands have also had high prevalence (48%) and has been observed in other surveillance efforts across North America (McKenzie et al., 2018; Haynes et al., 2022). Possibly, the most interesting finding was that *Nerodia sipedon* with *Ophidiomyces* were significantly less likely to exhibit clinical signs. Previous studies have found statistical associations with clinical signs and the likelihood of detection, including the first study on military lands (Allender et al., 2016; McKenzie et al., 2018; Chandler et al., 2019; Allender et al., 2020). In Kentucky, *Nerodia sipedon* that were positive were more likely to have skin lesions, thus differences seen in the current study may reflect a changing disease pathogenesis, regional differences, or differences in clinical sign evaluation.

It seems unlikely that differences in clinical sign evaluation was the cause of the lower *Nerodia* likelihood since skin lesions were not uncommonly observed in this study, in fact several animals with skin lesions were not detected with *Ophidiomyces* DNA (possible ophidiomycosis), thus contesting the thought that clinical signs were missed in *Nerodia*.

North American crotalids and *Nerodia* species have previously been shown to have a high prevalence of ophidiomycosis and may be uniquely sensitive to infection to due to their environment (*Nerodia*) or morphology (pits in crotalids) (Allender et al., 2015c; McBride et al., 2015; Lorch et al., 2016). Not surprisingly, with the exception of *Crotalus horridus*, most pitvipers sampled in this study had low prevalence similar to the last surveillance effort. It is possible that previous perceived susceptibility of crotalids represents a sampling bias, in which venomous species were sampled more frequently than non-venomous species.

Diagnosis was historically and in some cases continues to be the largest cause of variance in detection. Compared to the last project, the number of these negative snakes with skin lesions decreased, suggesting that biologists improved sample collection techniques during this phase of the project. 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).

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 New Mexico represents a new state for free-ranging snakes (captive snakes were identified in New Mexico previously). 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.

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 emerging 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 stakeholders to identify trends and new threats to both snakes and other wildlife species.

# MILITARY MISSION BENEFITS

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. ophidiicola*, 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, and electronic documents/protocols. In addition to field and lab results, military natural resource personnel have a better understanding of what signs to look for in snakes with ophidiomycosis, as well as how to conduct sampling for the disease. This leads to a better characterization of this threat to snakes.

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.

# LITERATURE CITED


Allender MC, Baker SJ, Wylie D, Loper D, Dreslik MJ, Phillips CA, *et al.* (2015a). Development of snake fungal disease after experimental challenge with *Ophidiomyces ophidiicola* in cottonmouths (*Agkistrodon piscivorous*). PloS one 10:e0140193.

Allender MC, Bunick D, Dzhaman E, Burrus L, and Maddox C (2015b). Development and use of a real-time polymerase chain reaction assay for the detection of Ophidiomyces ophidiicola in snakes. Journal of Veterinary Diagnostic Investigation 27:217-220.

Allender MC, Dreslik M, Wylie S, Phillips C, Wylie DB, Maddox C, Delaney MA, Kinsel M (2011) *Chrysosporium* sp. infection in Eastern Massasauga rattlesnakes. Emerging Infectious Diseases 17:2383–2384.

Allender MC, Hileman ET, Moore J, Tetzlaff S (2016) Detection of *Ophidiomyces*, the causative agent of snake fungal disease, in the Eastern massasauga (*Sistrurus catenatus*) in Michigan, USA, 2014. Journal of Wildlife Diseases 52:694-698.

Allender MC, Raudabaugh DB, Gleason FH, Miller AN (2015c) The natural history, ecology, and epidemiology of *Ophidiomyces ophidiicola* and its potential impact on free-ranging snake populations. Fungal Ecology 17:187–196.

Allender MC, Ravesi MJ, Haynes E, Ospina E, Petersen C, Phillips CA, et al. (2020) Ophidiomycosis, an emerging fungal disease of snakes: Targeted surveillance on military lands and detection in the western US and Puerto Rico. PLoS ONE 15(10): e0240415.

Aycrigg JL, Belote RT, Dietz MS, Aplet GH, Fischer RA. Bombing for biodiversity in the United States: response to Zentelis and Lindenmayer 2015. Conserv Lett. 2015; 8: 306–307.

Baker SJ, Haynes E, Gramhofer M, Stanford K, Bailey S, Christman M, Conley K, Frasca Jr S, Ossiboff RJ, Lobato D, Allender MC (2019) Case definition and diagnostic testing for snake fungal disease. Herpetological Review, in press.

Benton N, Ripley JD, Powledge F (eds.). Conserving Biodiversitly on Military Lands: A Guide for Natural Resource Managers. 2008. Arlington, Virginia: NatureServe.

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ, Ward B (2009) Bat white-nose syndrome: An emerging fungal pathogen? Science 323:227.

Burbrink FT, Lorch JM, Lips KR (2017) Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. Science Advances 3:e1701387.

Chongsuvivatwong V(2018) epiDisplay: Epidemiological Data Display Package. R version 3.5.0.1.

Clark RW, Marchand MN, Clifford BJ, Stechert R, Stephens S (2011) Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: interactions between climate change, disease, and loss of genetic diversity. Biological Conservation 144:886–891.

Crawford JA, Phillips CA, Peterman WE, MacAllister I, Wesslund NA, Kuhns AR, Dreslik MJ (2017) Chytrid infection dynamics in cricket frogs on military and public lands in the midwestern United States. Journal of Fish and Wildlife Management 8: 344-352.

Davy CM, Shirose L, Campbell D, Dillon R, McKenzie C, Nemeth N, Braithwaite T, Cai H, Degazio T, Dobbie T, Egan S, Fotherby H, Litzgus JD, Manorome P, Marks S, Paterson JE, Sigler L, Slavic D, Slavik E, Urquhart J, Jardine C. (2021) Revisiting ophidiomycosis (snake fungal disease) after a decade of targeted research. Frontiers in Veterinary Science 8:doi=10.3389/fvets.2021.665805.

Dolinski AC, Allender MC, Hsiao V, Maddox CM. 2014. Systemic *Ophidiomyces ophidiicola* infection in a free-ranging plains garter snake (*Thamnophis radix*). Journal of Herpetological Medicine and Surgery 24:7–10.

O'Dea MA, Jackson B, Jackson C, Xavier P, Warren K (2016) Discovery and Partial Genomic Characterisation of a novel nidovirus associated with respiratory disease in wild shingleback lizards (*Tiliqua rugosa*). PLoS One. 2016 Nov 9;11(11):e0165209. doi:10.1371/journal.pone.0165209. eCollection 2016.

Figueroa A, McKelvy AD, Grismer LL, Bell CD, Lailvaux SP (2016) A Species-Level Phylogeny of Extant Snakes with Description of a New Colubrid Subfamily and Genus. PLoS ONE 11(9): e0161070. https://doi.org/10.1371/journal.pone.0161070.

Franklinos LH, Lorch JM, Bohuski E, Fernandez JR, Wright ON, Fitzpatrick L, Petrovan S, Durrant C, Linton C, Bala´zˇ V, Cunningham AA (2017) Emerging fungal pathogen *Ophidiomyces ophidiicola* in wild European snakes. Scientific Reports 7:3844–3850.

Grace JB, Anderson TM, Seabloom EW, Borer ET, Adler PB, Harpole WS, *et al.* (2016) Integrative modelling reveals mechanisms linking productivity and plant species richness. Nature. 529: 390-393.

Haynes, E. (2021) Characterizing the epidemiology of ophidiomycosis in north american snakes through field studies, modeling, pathogen genomic analysis and treatment trials. PhD Dissertation, University of Illinois, pp. 227.

Hileman ET, Allender MC, Bradke DR, Faust LJ, Moore JA, Ravesi MJ, Tetzlaff SJ (2018) Estimation of *Ophidiomyce*s prevalence to evaluate snake fungal disease risk. The Journal of Wildlife Management 82(1):173–181.

Kabay E, Caruso NM, Lips K. (2013) Timber rattlesnakes may reduce incidence of Lyme disease in the Northeastern United States: Ecological Society of America Annual Conference, 98th, Minneapolis, Minnesota, August 4–9, 2013.

Ladner JT, Palmer JM, Ettinger CL, Stajich JE, Farrell TM, Glorioso BM, et al. (2022) The population genetics of the causative agent of snake fungal disease indicate recent introductions to the USA. PLoS Biol 20(6): e3001676. https://doi.org/10.1371/journal.pbio.3001676.

Lind M, McCoy CM, Farrell TM (2018) Tracking outcomes of snake fungal disease in free-ranging pygmy rattlesnakes (*Sistrurus catenatus*). Journal of Wildlife Diseases 54:352-356.

Lorch JM, Knowles S, Lankton JS, Michell K, Edwards JL, Kapfer JM, Staffen RA, Wild ER, Schmidt KZ, Ballmann AE, Blodgett D, Farrell TM, Glorioso BM, Last LA, Price SJ, Schuler KL, Smith CE, Jr Wellehan JFX, Blehert DS (2016) Snake fungal disease: an emerging threat to wild snakes. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150457.

Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS (2015) Experimental infection of snakes with *Ophidiomyces ophidiicola* causes pathological changes that typify snake fungal disease. mBio 6:1–9.

Lorch JM, Price SJ, Lankton JS, Drayer AN (2021) Confirmed cases of Ophidiomycosis in museum specimens from as early as1945. United States Emerging Infectious Diseases. https://doi.org/10.3201/eid2707.204864 .

Lovich, R.E., C. Petersen, A. Dalsimer. 2015. Department of Defense Natural Resources Program. Strategic Plan for Amphibian and Reptile Conservation and Management on Department of Defense Lands. U.S. Department of Defense, city, state Washington, D.C., USA. 14 pp.

Mazerolle MJ. 2017. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.1-1.

McBride MP, Wojick KB, Georoff TA, imbro J, Garner MM, Wang X, Childress AL, Wellehan JFX (2015) *Ophidiomyces ophidiicola* dermatitis in eight free-ranging timber rattlesnakes (*Crotalus horridus*) from Massachusetts. Journal of Zoo and Wildlife Medicine 46:86-94.

McCoy CM, Lind CM, Farrell TM (2017) Environmental and physiological correlates of the severity of clinical signs of snake *Ophidiomyces ophidiicola* diagnostics and seasonality fungal disease in a population of pigmy rattlesnakes, *Sistrurus miliarius*. Conservation Physiology 5:1–10.

McKenzie JM, Price SJ, Fleckenstein JL, Drayer AN, Connette GM, Bohuski E, Lorch JM. (2018) Field Diagnostics and Seasonality of *Ophidiomyces ophidiicola* in Wild Snake Populations. Ecohealth 16:141-150.

Origgi FC, Pisano SRR, Glaizot O, Hertwig ST, Schmitz A, Ursenbacher S. (2022) Ophidiomyces ophiodiicola, etiologic agent of snake fungal disease, in Europe since late 1950s. Emerging Infectious Diseases 28(1): 2064-2068.

Ostfeld RS and Holt RD (2004) Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. Frontiers in Ecology and the Environment 2:13-20.

Petersen CE, Lovich RE, Stallings S (2018). Amphibians and Reptiles of United States Department of Defense Installations. Herpetological Conservation and Biology 13(3):652–661.

Pontremoli C, Forni D, Cagliani R, Sironi M (2018) Analysis of Reptarenavirus genomes indicates different selective forces acting on the S and L segments and recent expansion of common genotypes. Infect Genet Evol. 2018 Oct;64:212-218. doi: 10.1016/j.meegid.2018.06.031. Epub 2018 Jun 30.

Robertson J, Chinnadurai SK, Woodburn DB, Adkesson MJ, Landolfi JA (2016) Disseminated Ophidiomyces ophidiicola infection in a captive eastern massasauga (*Sistrurus catenatus catenatus*). Journal of Zoo and Wildlife Medicine 47:337-40.

Rzadkowska M., Allender MC, O'Dell M., Maddox C. (2016) Evaluation of common disinfectants effective against *Ophidiomyces ophidiicola*, the causative agent of snake fungal disease. Journal of Wildlife Disease 52:759–762.

Sigler L, Hambleton S, Pare JA (2013) Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. Journal of Clinical Microbiology 51:3338–3357.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, et al. (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth. 4:125–34. doi:10.1007/s10393-007-0093-5.

Stein BA, Scott C, Benton N. 2008. Federal lands and endangered species: the role of military and other federal lands in sustaining biodiversity. Bioscience 58:339.347.

Takami Y, Nam K-O, Takai Y, Kadekaru S, Hemmi C, Hosoya T, Une Y. (2021) First report of ophidiomycosis in Asia caused by Ophidiomyces ophiodiicola in captive snakes in Japan. The Journal of Veterinary Medical Science 83(8): 1234-1239.

Tetzlaff SJ, Ravesi MJ, Allender MC, Carter ET, DeGregorio, Jasiomovich JM, Kingsbury BA (2018) Snake fungal disease affects behavior of free-ranging massasauga rattlesnakes (*Sistrurus catenatus*). Herpetological Conservation and Biology 12:624-634.

Wilson EB (1927). Probable inference, the law of succession, and statistical inference. Journal of the American Statistical Association. 22: 209–212.

Table 1. Sample sizes and results of *Ophidiomyces* testing on military installations, by state, where snakes were sampled in 2021-2022 that were included in multi-variable model. Sites that had fewer than 5 samples submitted are not listed here.

| Installation.Name | State | Negative | Positive | Prevalence |
|---|---|---|---|---|
| Army National Guard - McCrady Training Center | South Carolina | 14 | 1 | 7% |
| Arnold AFB | Tennessee | 10 | 10 | 50% |
| Camp Blanding Joint Training Center | Florida | 5 | 0 | 0% |
| Camp Grayling | Michigan | 100 | 0 | 0% |
| Camp Lejeune | North Carolina | 6 | 0 | 0% |
| Camp Pendleton | California | 8 | 0 | 0% |
| Camp Ripley Training Center | Minnesota | 6 | 0 | 0% |
| Conchas Dam and Lake Project | New Mexico | 3 | 1 | 25% |
| Fort  Wolters | Texas | 37 | 1 | 3% |
| Fort Custer Training Center | Michigan | 3 | 0 | 0% |
| Fort Drum | New York | 12 | 0 | 0% |
| Fort Gordon Resources Branch | Georgia | 0 | 3 | 100% |
| Fort Hunter Legget | California | 2 | 0 | 0% |
| Fort Knox | Kentucky | 4 | 6 | 60% |
| Fort Leavenworth | Kansas | 22 | 7 | 24% |
| Fort Lee | Virginia | 20 | 27 | 57% |
| Fort McCoy | Wisconsin | 37 | 6 | 14% |
| Fort Polk | Louisiana | 9 | 1 | 10% |
| Fort Stewart | Georgia | 3 | 15 | 83% |
| Hurlburt Field | Florida | 10 | 1 | 9% |
| Idaho Army National Guard | Idaho | 32 | 0 | 0% |
| Joint Base Langley-Eustis | Virginia | 47 | 7 | 13% |
| Joint Base McGuire-Dix-Lakehurst | New Jersey | 8 | 0 | 0% |
| Massachusetts | Massachusetts | 1 | 0 | 0% |
| MCAS Cherry Point | North Carolina | 1 | 0 | 0% |
| MCB Camp Lejeune | North Carolina | 5 | 7 | 58% |
| MCAS Miramar | California | 1 | 0 | 0% |
| Mississippi National Guard | Mississippi | 23 | 2 | 8% |
| Moody AFB | Georgia | 2 | 1 | 33% |
| NAS Fallon | Arizona | 15 | 0 | 0% |
| NAS Oceana | Virginia | 9 | 1 | 10% |
| NAS Whidbey Is. | Washington | 10 | 0 | 0% |
| Naval Support Facility Dahlgren | Virginia | 1 | 2 | 67% |
| Naval Weapons Station Yorktown | Virginia | 14 | 13 | 48% |
| New Boston | New Hampshire | 18 | 5 | 22% |
| New Hampshire Army National Guard | New Hampshire | 2 | 1 | 33% |
| North Dakota National Guard | North Dakota | 3 | 0 | 0% |
| NSA Crane | Indiana | 3 | 2 | 40% |
| Oklahoma National Guard | Oklahoma | 11 | 0 | 0% |

| | | | | |
|---|---|---|---|---|
| Oregon Army National Guard | Oregon | 5 | 0 | 0% |
| PA Dept. of Veterans and Military Affairs | Pennsylvania | 11 | 17 | 61% |
| Pinion Canyon | Colorado | 3 | 0 | 0% |
| Tinker Air Force Base | Oklahoma | 9 | 0 | 0% |
| Utah Army National Guard | Utah | 40 | 0 | 0% |
| Vandenberg Air Force Base | California | 9 | 0 | 0% |
| West Point | New York | 21 | 15 | 42% |
| Wright Patterson AFB | Ohio | 21 | 6 | 22% |
| Yakima Training Center | Washington | 31 | 0 | 0% |
| TOTAL | | 668 | 158 | 19% |

Table 2. Ophidiomycosis results by species for snakes assayed for *Ophidiomyces ophidiicola* on military installations from 2021-2022 that were included in multi-variable model. Species that had fewer than 5 samples submitted are not listed here.

| Species | Negative | Ophidiomyces Present | Possible Ophidiomycosis | Apparent Ophidiomycosis | Total |
|---|---|---|---|---|---|
| *Agkistrodon contortrix* | 5 | 2 | 0 | 1 | 8 |
| *Agkistrodon piscivorus* | 5 | 2 | 0 | 0 | 7 |
| *Carphophis amoenus* | 3 | 2 | 0 | 1 | 6 |
| *Charina bottae* | 1 | 0 | 0 | 0 | 1 |
| *Coluber constrictor* | 17 | 6 | 4 | 15 | 42 |
| *Coluber constrictor constrictor* | 23 | 3 | 2 | 6 | 34 |
| *Coluber constrictor flaviventris* | 4 | 0 | 0 | 0 | 4 |
| *Coluber constrictor priapus* | 0 | 0 | 0 | 2 | 2 |
| *Coralus scutulatus* | 2 | 0 | 0 | 0 | 2 |
| *Crotalus adamanteus* | 1 | 0 | 0 | 0 | 1 |
| *Crotalus atrox* | 7 | 0 | 0 | 0 | 7 |
| *Crotalus horridus* | 3 | 2 | 1 | 1 | 7 |
| *Crotalus lepidus* | 3 | 0 | 0 | 0 | 3 |
| *Crotalus molossus* | 2 | 0 | 0 | 0 | 2 |
| *Crotalus oreganus* | 7 | 0 | 0 | 0 | 7 |
| *Crotalus oreganus helleri* | 2 | 0 | 0 | 0 | 2 |
| *Crotalus oreganus lutosus* | 37 | 0 | 0 | 0 | 37 |
| *Crotalus ruber* | 6 | 0 | 1 | 0 | 7 |
| *Diadophis punctatus* | 25 | 2 | 1 | 4 | 32 |
| *Diadophis punctatus edwardsii* | 1 | 0 | 0 | 0 | 1 |
| *Drymarchon couperi* | 0 | 3 | 0 | 12 | 15 |
| *Haldea striatula* | 6 | 0 | 0 | 1 | 7 |
| *Heterdon platirhinos* | 0 | 0 | 1 | 0 | 1 |
| *Heterodon platirhinos* | 10 | 0 | 2 | 1 | 13 |
| *Heterodon simus* | 0 | 1 | 0 | 0 | 1 |
| *Lampropeltis californiae* | 1 | 0 | 0 | 0 | 1 |
| *Lampropeltis calligaster* | 1 | 0 | 1 | 0 | 2 |
| *Lampropeltis elapsoides* | 5 | 4 | 1 | 0 | 10 |
| *Lampropeltis getula* | 4 | 1 | 0 | 1 | 6 |
| *Lampropeltis holbrooki* | 2 | 1 | 0 | 0 | 3 |
| *Lampropeltis nigra* | 0 | 0 | 1 | 2 | 3 |
| *Lampropeltis triangelem* | 1 | 0 | 0 | 0 | 1 |
| *masticophis flagellum* | 2 | 0 | 0 | 0 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| *Masticophis flagellum* | 14 | 1 | 1 | 2 | 18 |
| *Masticophis flagellum testaceus* | 1 | 0 | 0 | 0 | 1 |
| *Masticophis taeniatus* | 1 | 0 | 0 | 0 | 1 |
| *Nerodia erthrogaster* | 1 | 0 | 0 | 0 | 1 |
| *Nerodia erythrogaster* | 4 | 1 | 0 | 0 | 5 |
| *Nerodia fasciata* | 6 | 0 | 2 | 2 | 10 |
| *Nerodia rhombifer* | 1 | 0 | 0 | 0 | 1 |
| *Nerodia sipedon* | 11 | 13 | 2 | 4 | 30 |
| *Nerodia taxispilota* | 0 | 0 | 0 | 1 | 1 |
| *Opheodrys aestivus* | 10 | 1 | 2 | 1 | 14 |
| *Opheodrys vernalis* | 6 | 0 | 6 | 0 | 12 |
| *Pantherophis alleghaniensis* | 19 | 4 | 10 | 6 | 39 |
| *Pantherophis gloydi* | 0 | 1 | 1 | 1 | 3 |
| *Pantherophis guttatus* | 9 | 1 | 0 | 0 | 10 |
| *Pantherophis melanoleucus* | 0 | 2 | 0 | 0 | 2 |
| *Pantherophis obsoletus* | 25 | 4 | 5 | 3 | 37 |
| *Pantherophis slowenskii* | 0 | 0 | 1 | 0 | 1 |
| *Pantherophis spiloides* | 10 | 2 | 0 | 3 | 15 |
| *Pantherophis vulpinus* | 0 | 0 | 1 | 0 | 1 |
| *Pitiuphis ruthveni* | 1 | 0 | 0 | 0 | 1 |
| *Pituophis cantenifer* | 15 | 0 | 0 | 0 | 15 |
| *Pituophis catenifer deserticola* | 24 | 0 | 0 | 0 | 24 |
| *Pituophis catenifer sayi* | 29 | 3 | 10 | 1 | 43 |
| *Pituophis melanoleucus* | 1 | 1 | 0 | 0 | 2 |
| *Pituophis melanoleucus lodingi* | 2 | 0 | 0 | 0 | 2 |
| *Pituophis ruthveni* | 1 | 0 | 0 | 0 | 1 |
| *Regina grahamii* | 1 | 0 | 0 | 0 | 1 |
| *Regina rigida* | 1 | 0 | 0 | 0 | 1 |
| *Rena dulcis* | 1 | 0 | 1 | 0 | 2 |
| *Salvadora grahamiae* | 1 | 0 | 0 | 0 | 1 |
| *sistrurus catenatus* | 3 | 0 | 0 | 0 | 3 |
| *Sistrurus catenatus* | 97 | 0 | 2 | 0 | 99 |
| *Sonora semiannulata* | 4 | 0 | 0 | 0 | 4 |
| *Storeria dekayi* | 8 | 2 | 1 | 2 | 13 |
| *Storeria occipitomaculata* | 6 | 0 | 1 | 0 | 7 |
| *Storeria occipitumocalata* | 1 | 0 | 0 | 0 | 1 |
| *Tantilla gracilis* | 1 | 0 | 0 | 0 | 1 |
| *Thamnophis elegans* | 1 | 0 | 1 | 0 | 2 |
| *Thamnophis ordinoides* | 6 | 0 | 0 | 0 | 6 |
| *Thamnophis proximus* | 5 | 0 | 0 | 1 | 6 |

| | | | | | |
|---|---|---|---|---|---|
| *Thamnophis proximus proximus* | 1 | 0 | 0 | 0 | 1 |
| *Thamnophis proximus rubrilineatus* | 1 | 0 | 0 | 0 | 1 |
| *Thamnophis sauritus* | 1 | 0 | 0 | 1 | 2 |
| *Thamnophis sauritus sauritus* | 3 | 0 | 0 | 0 | 3 |
| *Thamnophis sirtalis* | 63 | 8 | 10 | 8 | 89 |
| *Thamnophis sirtalis infernalis* | 5 | 0 | 0 | 0 | 5 |
| *Virginia valeriae* | 1 | 0 | 0 | 1 | 2 |
| Total | 596 | 74 | 72 | 84 | 826 |

Figure 1. Spatial distribution of *Ophidiomyces ophidiicola* detection in snakes on military installations sampled in 2021-2022. White = states not sampled, blue = states with no detection of *O. ophidiicola*, orange = states detected with *O. ophidiicola*