DoD Environmental Planning and Conservation Webinar Series

DoD Snake Fungal Disease Survey, Pt. II (Legacy Project NR-21-005)

October 12, 2023

Please mute your phones

Audio Dial-In: 410-874-6749

Participant Code: 971-151-440#

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Ophidiomycosis on Military Lands: Part III

WILDLIFE
EPIDEMIOLOGY LABORATORY
vetmed.illinois.edu/wel

Robert E. Lovich, Matthew C. Allender, Emilie Ospina,
Christopher E. Petersen

Etiology

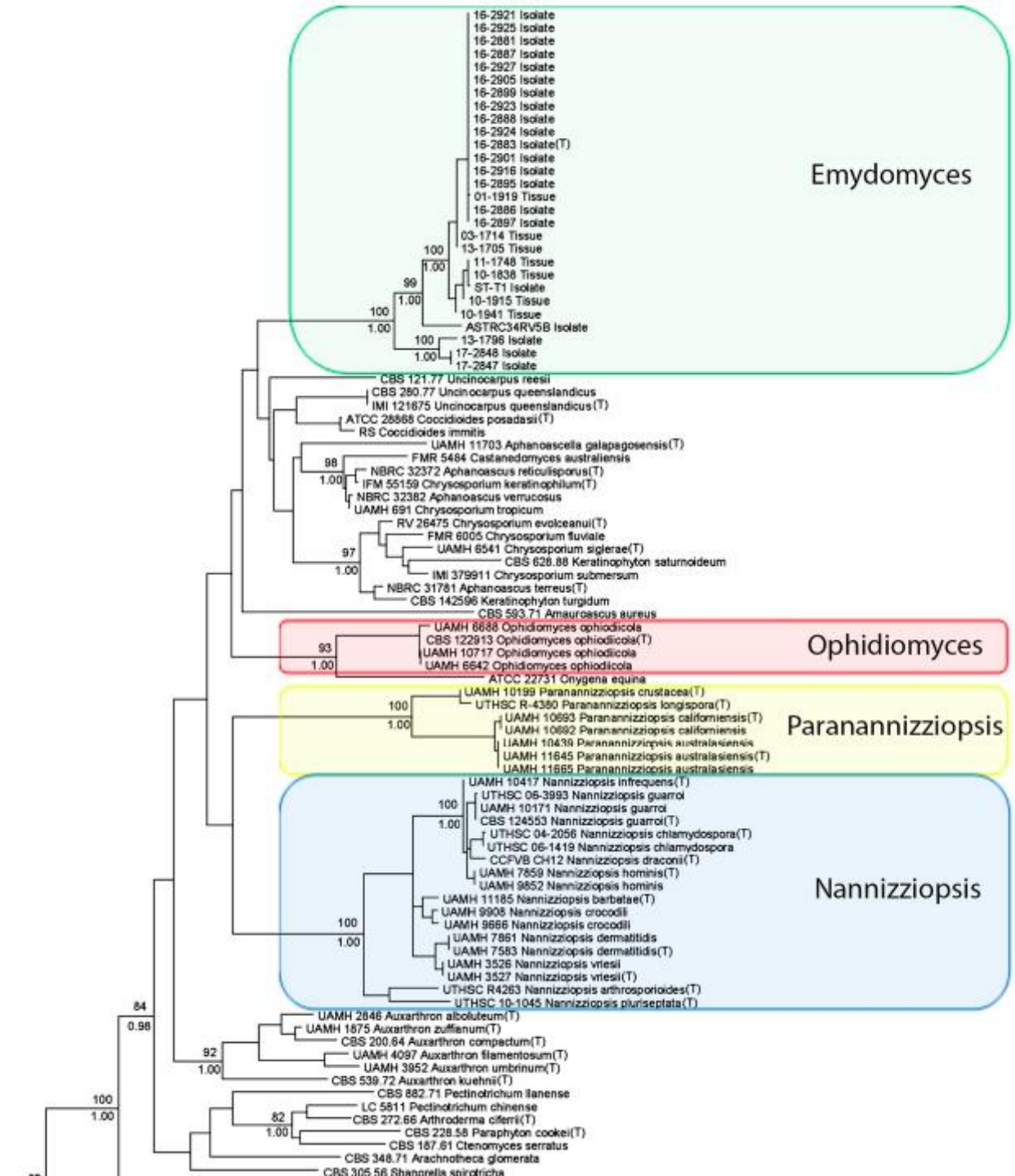
Ophidiomyces ophidiicola

- Order Onygenales
  - Closely related to other reptile skin pathogens
- Previous names:
  - *Chrysosporium ophiodiicola*
  - CANV
  - CANV-like

Nannizziopsis spp.

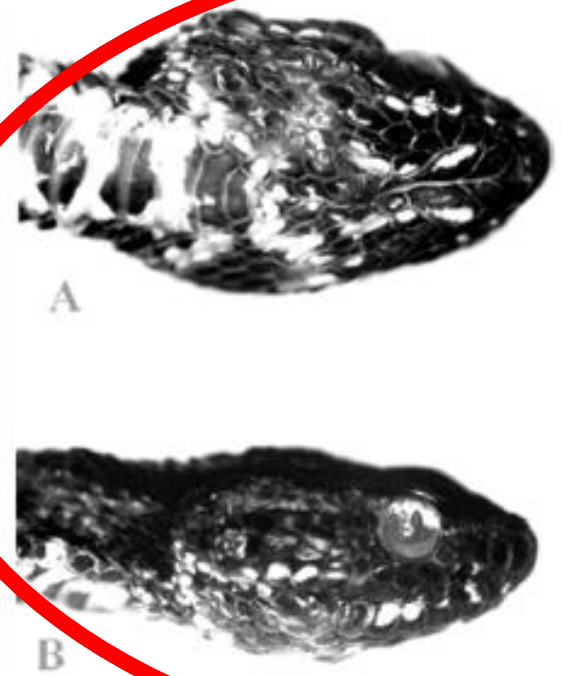
Paranannizziopsis spp.

Emydomyces testavorans


16-2921 Isolate
16-2925 Isolate
16-2881 Isolate
16-2887 Isolate
16-2927 Isolate
16-2905 Isolate
16-2899 Isolate
16-2923 Isolate
16-2888 Isolate
16-2924 Isolate
16-2883 Isolate(T)
16-2901 Isolate
16-2916 Isolate
16-2895 Isolate
01-1919 Tissue
16-2886 Isolate
16-2897 Isolate
03-1714 Tissue
13-1705 Tissue
11-1748 Tissue
10-1838 Tissue
ST-T1 Isolate
10-1915 Tissue
10-1941 Tissue
ASTRC34RV5B Isolate
13-1796 Isolate
17-2848 Isolate
17-2847 Isolate
Emydomyces
CBS 121.77 Uncinocarpus reesii
CBS 280.77 Uncinocarpus queenslandicus
IMI 121675 Uncinocarpus queenslandicus(T)
ATCC 28868 Coccidioides posadasii(T)
RS Coccidioides immitis
UAMH 11703 Aphanoascella galapagosensis(T)
FMR 5484 Castanedomyces australiensis
NBRC 32372 Aphanoascus reticulisporus(T)
IFM 55159 Chrysosporium keratinophilum(T)
NBRC 32382 Aphanoascus verrucosus
UAMH 691 Chrysosporium tropicum
RV 26475 Chrysosporium evolceanui(T)
FMR 6005 Chrysosporium fluviale
UAMH 6541 Chrysosporium siglerae(T)
CBS 628.88 Keratinophyton saturnoideum
IMI 379911 Chrysosporium submersum
NBRC 31781 Aphanoascus terreus(T)
CBS 142596 Keratinophyton turgidum
CBS 593.71 Amauroascus aureus
UAMH 6688 Ophidiomyces ophiodicola
CBS 122913 Ophidiomyces ophiodicola(T)
UAMH 10717 Ophidiomyces ophiodicola
UAMH 6642 Ophidiomyces ophiodicola
Ophidiomyces
ATCC 22731 Onygena equina
UAMH 10199 Paranannizziopsis crustacea(T)
UTHSC R-4380 Paranannizziopsis longispora(T)
UAMH 10693 Paranannizziopsis californiensis(T)
UAMH 10692 Paranannizziopsis californiensis
UAMH 10438 Paranannizziopsis australasiensis
UAMH 11645 Paranannizziopsis australasiensis(T)
UAMH 11665 Paranannizziopsis australasiensis
Paranannizziopsis
UAMH 10417 Nannizziopsis infrequens(T)
UTHSC 06-3993 Nannizziopsis guarroi
UAMH 10171 Nannizziopsis guarroi
CBS 124553 Nannizziopsis guarroi(T)
UTHSC 04-2056 Nannizziopsis chlamydospora(T)
UTHSC 06-1419 Nannizziopsis chlamydospora
CCFVB CH12 Nannizziopsis draconii(T)
UAMH 7859 Nannizziopsis hominis(T)
UAMH 9852 Nannizziopsis hominis
UAMH 11185 Nannizziopsis barbatae(T)
UAMH 9908 Nannizziopsis crocodili
UAMH 9666 Nannizziopsis crocodili
UAMH 7851 Nannizziopsis dermatitidis
UAMH 7583 Nannizziopsis dermatitidis(T)
UAMH 3526 Nannizziopsis vriesii
UAMH 3527 Nannizziopsis vriesii(T)
UTHSC R4263 Nannizziopsis arthrosporioides(T)
UTHSC 10-1045 Nannizziopsis pluriseptata(T)
Nannizziopsis
UAMH 2846 Auxarthron alboluteum(T)
UAMH 1875 Auxarthron zuffianum(T)
CBS 200.64 Auxarthron compactum(T)
UAMH 4097 Auxarthron filamentosum(T)
UAMH 3952 Auxarthron umbrinum(T)
CBS 539.72 Auxarthron kuehnii(T)
CBS 882.71 Pectinotrichum llanense
LC 5811 Pectinotrichum chinense
CBS 272.66 Arthroderma ciferrii(T)
CBS 228.58 Paraphyton cookei(T)
CBS 187.61 Ctenomyces serratus
CBS 348.71 Arachnotheca glomerata
CBS 305.56 Shanorella spiralitricha

AN OUTBREAK OF FUNGAL DERMATITIS AND STOMATITIS IN A FREE-RANGING POPULATION OF PIGMY RATTLESNAKES (*SISTRURUS MILIARIUS BARBOURI*) IN FLORIDA

Joseph L. Cheatwood,[1,4] Elliott R. Jacobson,[1] Peter G. May,[2] Terence M. Farrell,[2]
Bruce L. Homer,[1] Don A. Samuelson,[1] and James W. Kimbrough[3]



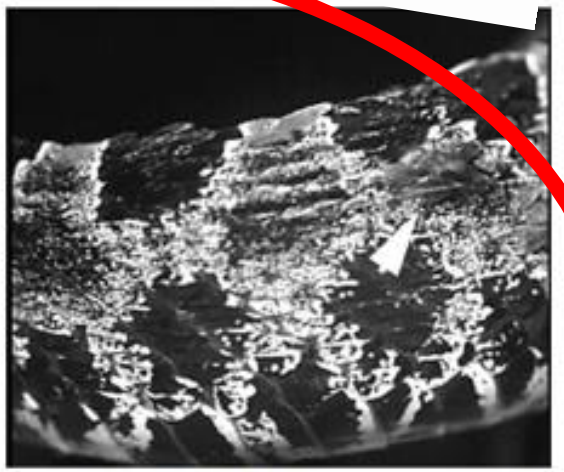
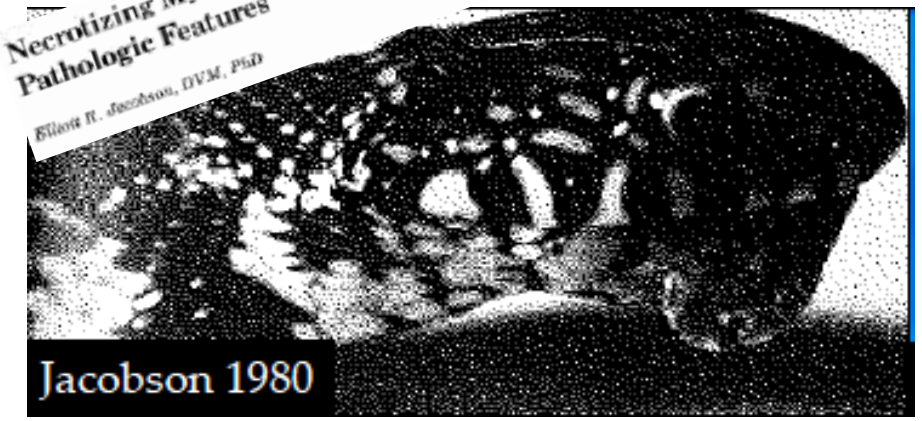


FIGURE 2. Focal epidermal necrosis and subcutaneous masses (arrow) in the skin of a pigmy rattlesnake.

et al., 1996). Between Februar[illegible]992 and September 1997 59 pigmy rattlesnakes

Necrotizing Mycotic Dermatitis in Snakes: Clinical and Pathologic Features

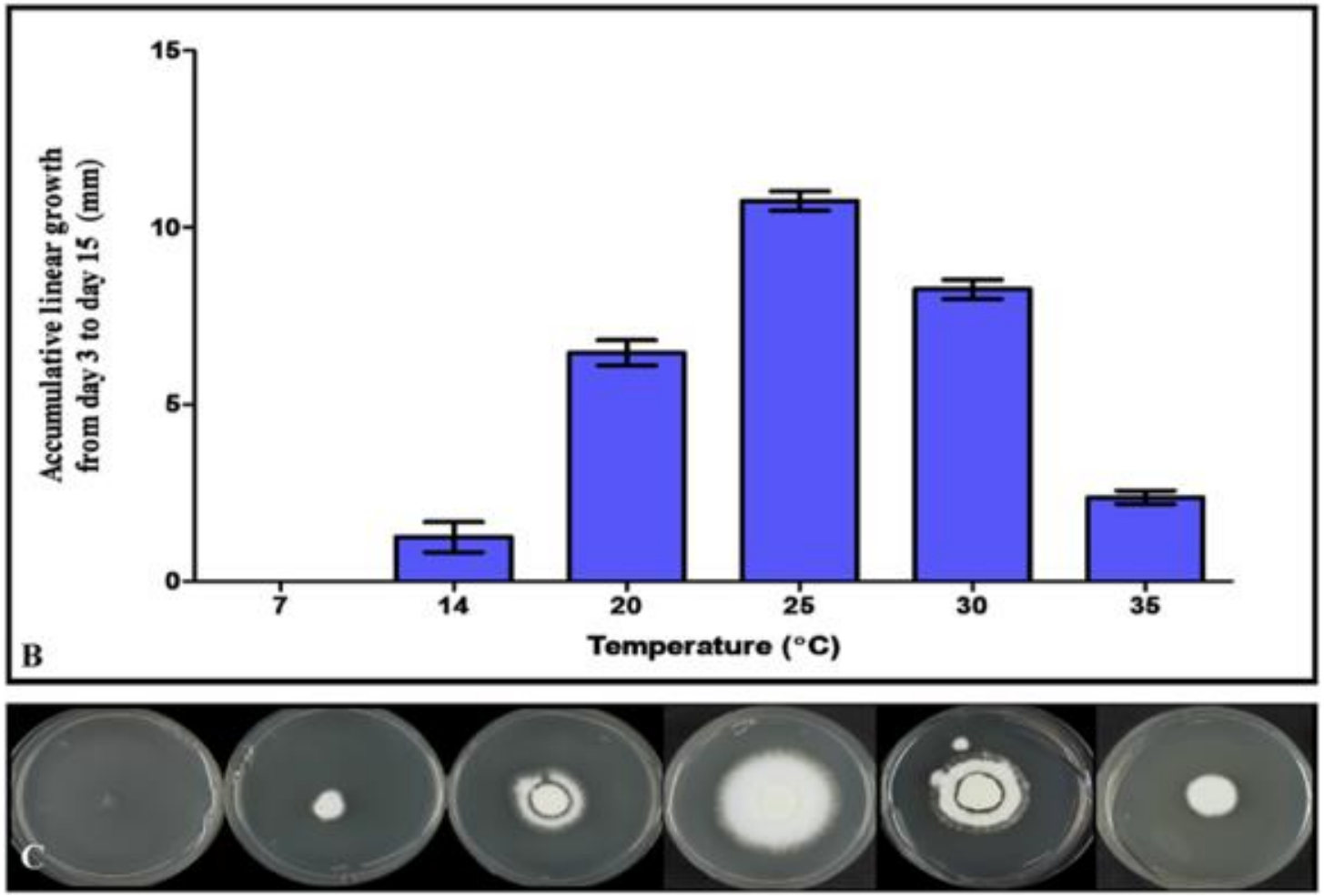
Elliott R. Jacobson, DVM, PhD



Jacobson 1980

Isolation and Characterization of a New Fungal Species, *Chrysosporium ophiodiicola*, from a Mycotic Granuloma of a Black Rat Snake (*Elaphe obsoleta obsoleta*)[V]

S. Rajeev,[1]* D. A. Sutton,[2] B. L. Wickes,[3] D. L. Miller,[1] D. Giri,[2] M. Kane Meyer,[5] E. H. Thompson,[2] M. G. Rinaldi,[2] A. M. Romano[illegible] J. Cano,[4] and J. Guarro[4]



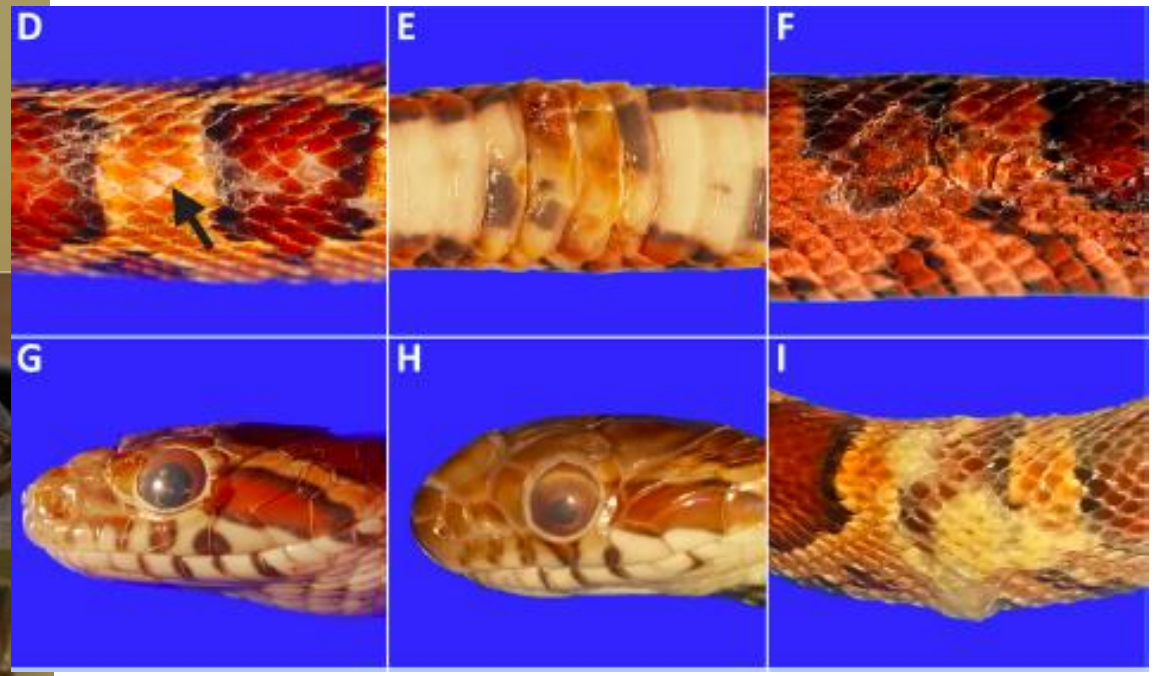
- Robust growth
  - Dead fish
  - Dead insect
  - Dead mushroom
  - Deminearlized shrimp exoskeletons
- Sparse growth
  - Demineralized-deproteinated exoskeletons

Development of Snake Fungal Disease after Experimental Challenge with *Ophidiomyces ophiodiicola* in Cottonmouths (*Agkistrodon piscivorous*)

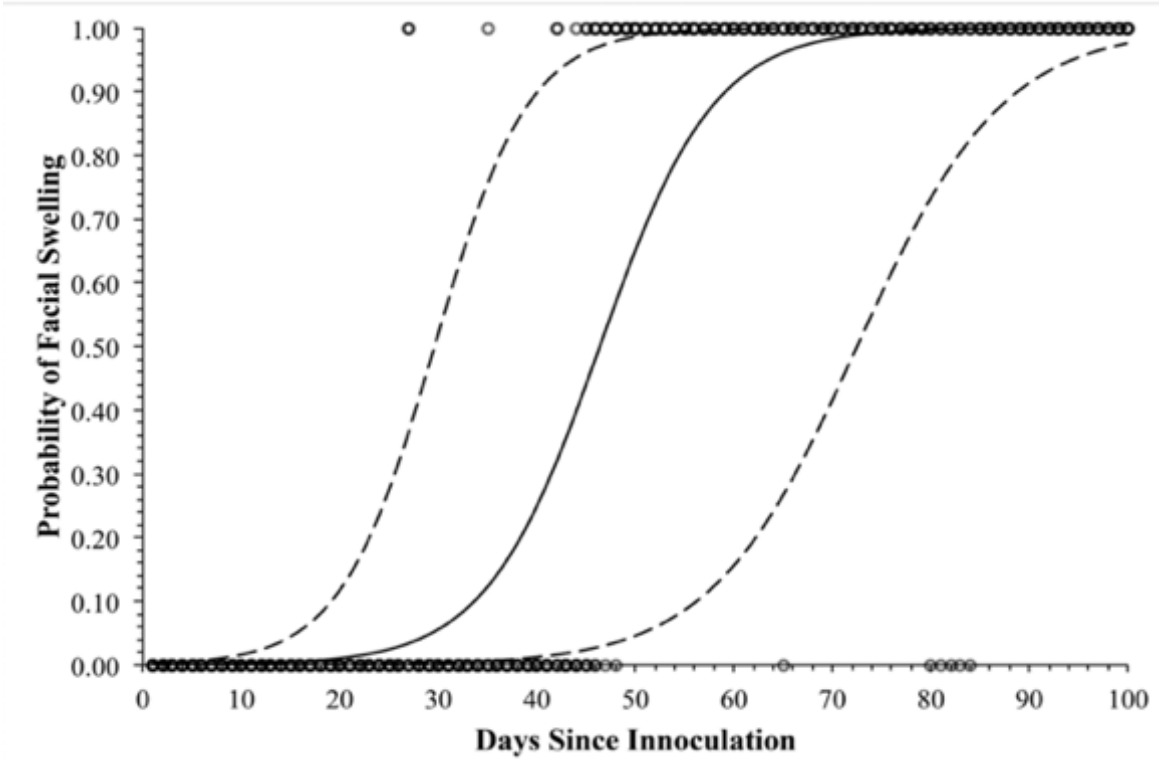
Matthew C. Allender[1,2,*], Sarah Baker[2], Daniel Wylie[2], Daniel Loper[1], Michael J. Dreslik[3], Christopher A. Phillips[3], Carol Maddox[3], Elizabeth A. Driskell[3]

Experimental Infection of Snakes with *Ophidiomyces ophiodiicola* Causes Pathological Changes That Typify Snake Fungal Disease

Jeffrey M. Lorch,[a] Julia Lankton,[a] Katrien Werner,[a] Elizabeth A. Falendysz,[a] Kevin McCurley,[b] David S. Blehert[a]

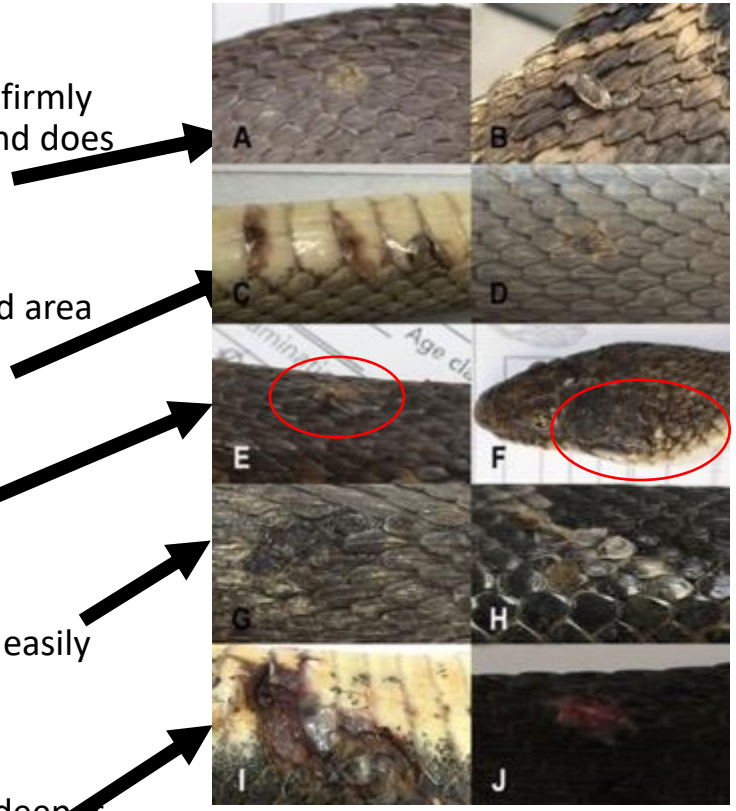


Development of clinical signs



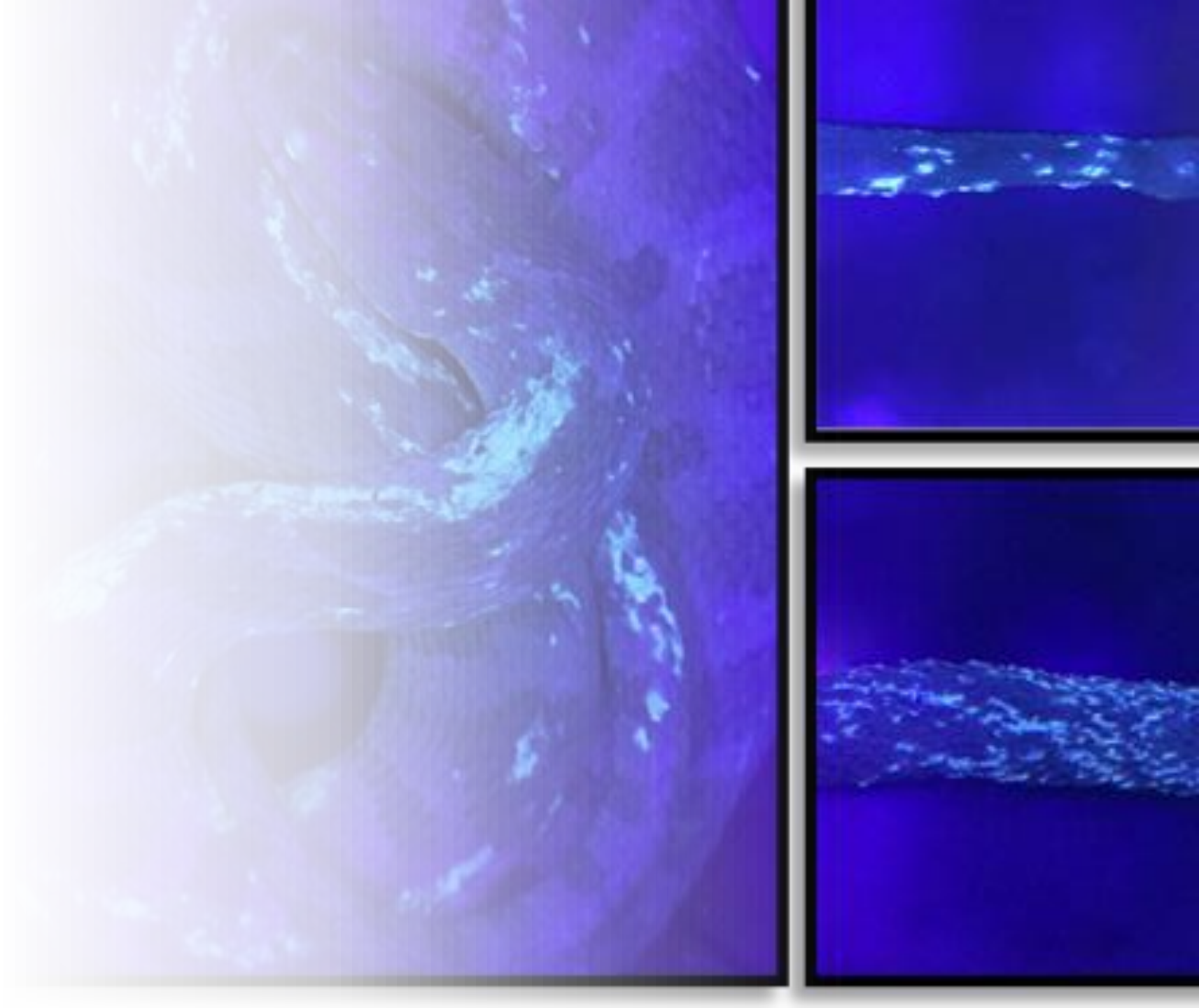
Physical examination

- Displaced/thickened scale(s)
  - Scale that appears elevated or misaligned with adjacent scales but is firmly attached to underlying dermis. The scale is not typically discolored and does not slough when light pressure is applied.
- Necrotic scale
  - Similar to above, but discoloration is usually present, and the affected area sloughs when light pressure is applied.
- Granuloma:
  - localized cutaneous or subcutaneous swelling and necrosis often accompanied by discoloration.
- Scab/crust:
  - Collection of adjacent hardened, thickened, or necrotic scales that is easily removed en bloc when light pressure is applied.
- Ulcer:
  - Complete loss of scales/epidermis that expose underlying dermis or deeper tissues.



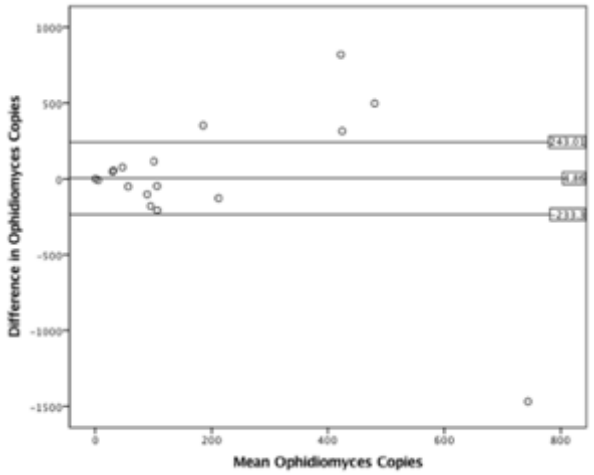
Ophidiomycosis Diagnosis

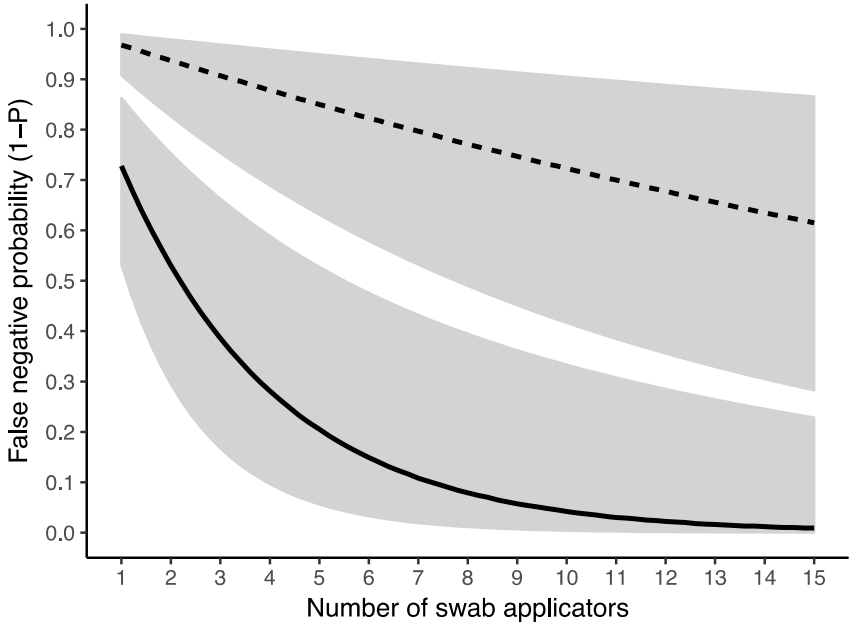
- qPCR – most common, sensitive
- Culture
- Biopsy
- No antibody tests
- UV light



Molecular diagnostics

- Tissue invasion prevents surface sampling from being effective
- Focal distribution may allow for specific tissue collection to miss infection
- Swabs of clinical animals were shown to have nearly 8 times higher detection probability than swabs of “healthy” appearing skin
  - 100% agreement in paired swab and biopsy samples
  - False negative rate is high with sampling, but reduced with multiple swabs

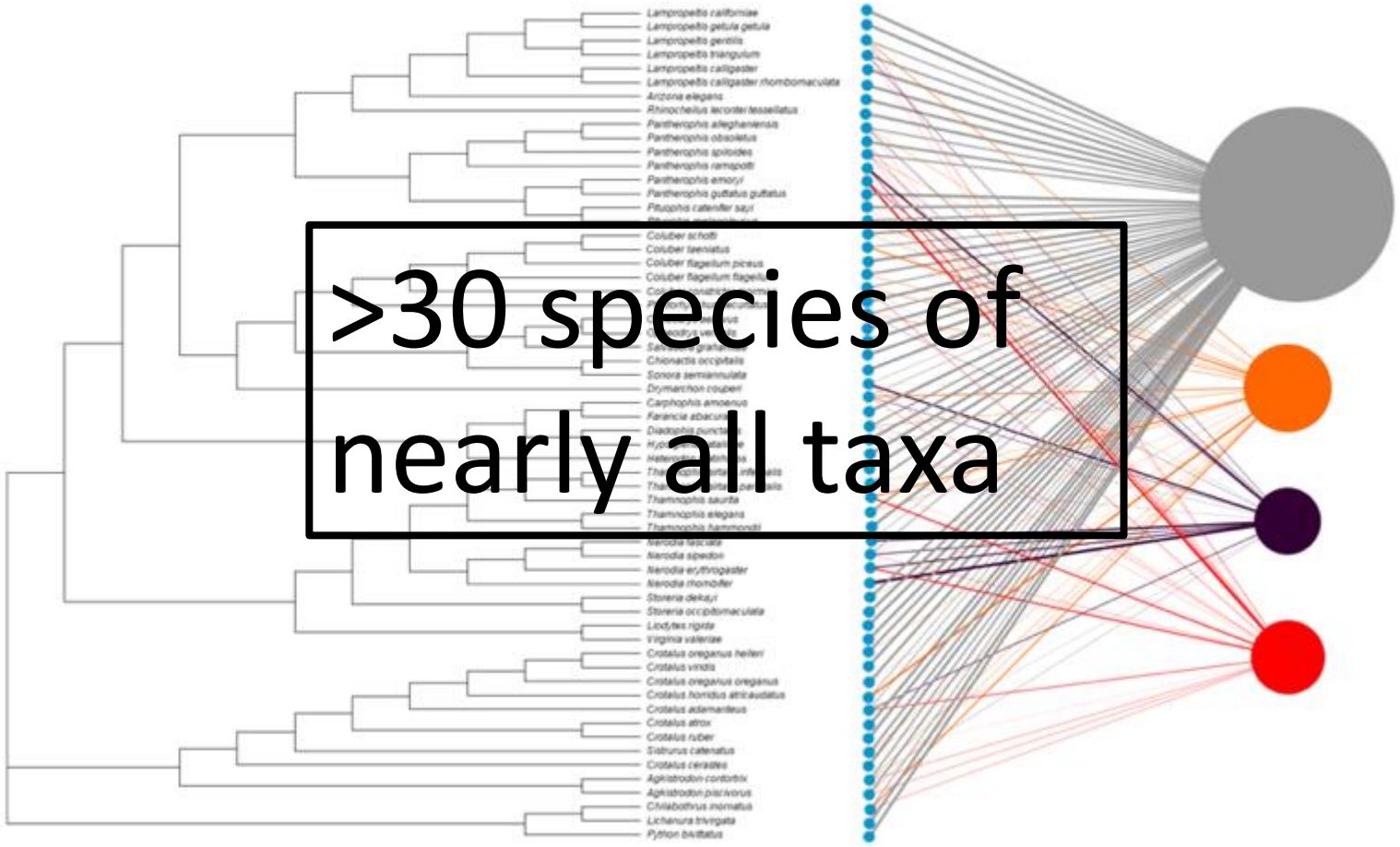




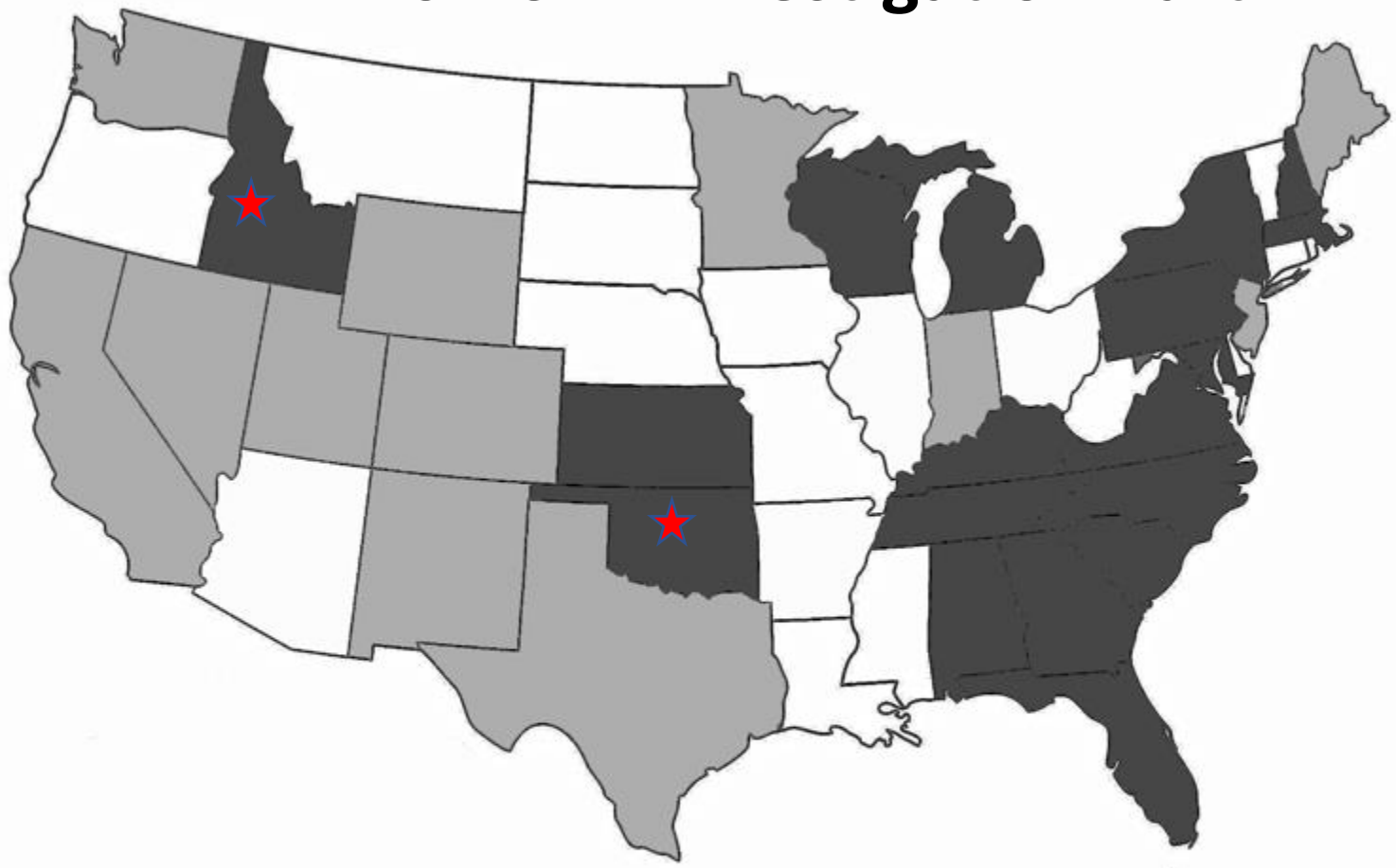
Ophidiomycosis Categories (Baker et al. 2019)

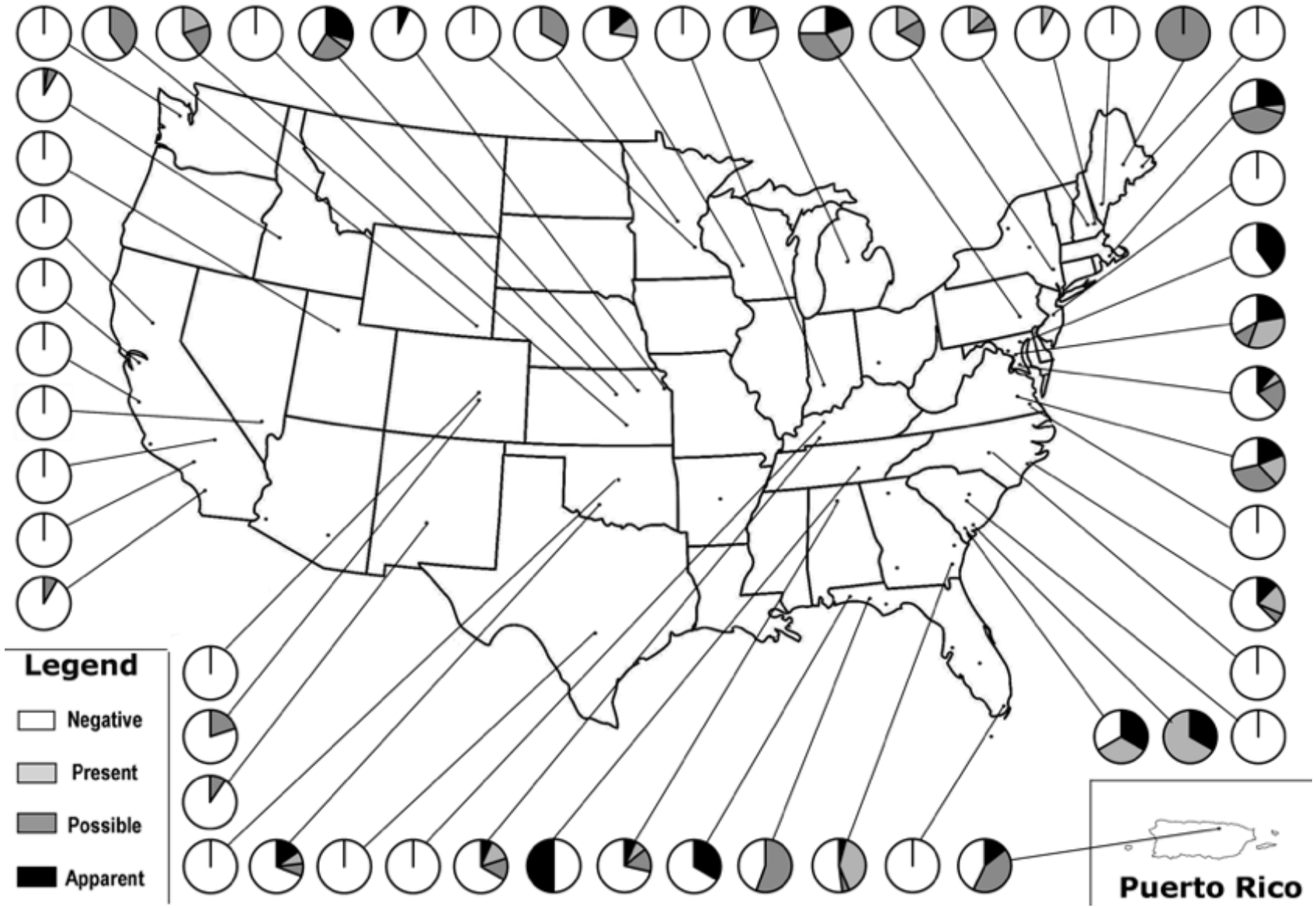
	Category	Lesion presence	qPCR result	Histopathologic findings
1.	Negative	No	Negative	No abnormal findings.
2.	Ophidiomyces present	No	Positive	No abnormal findings.
3.	Possible ophidiomycosis	Yes	Negative	No intralesional fungi present OR intralesional fungi present but no arthroconidia.
4.	Apparent ophidiomycosis	Yes	Positive	No intralesional fungi present OR intralesional fungi present but no arthroconidia.
5.	Confirmed ophidiomycosis	Yes	Positive	Intralesional fungal hyphae and arthroconidia present.

Taxon Distribution



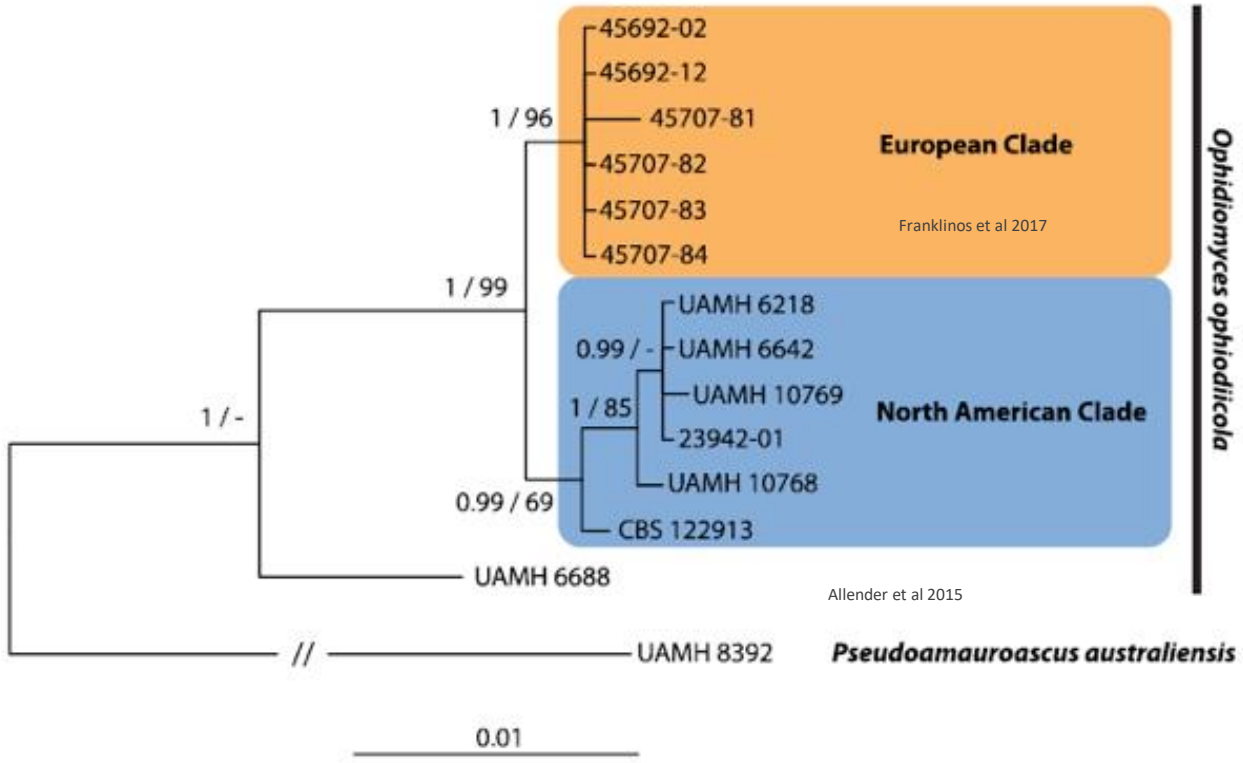
DoD SFD investigation Part 1




Legend
Negative
Present
Possible
Apparent
Puerto Rico

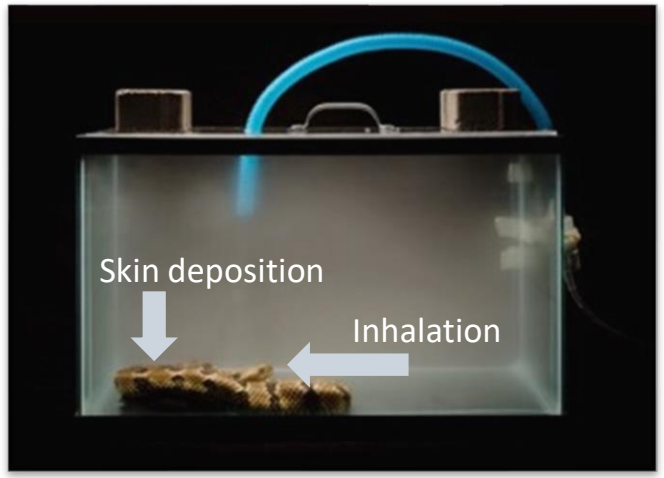
O. ophidiicola Genetic Diversity

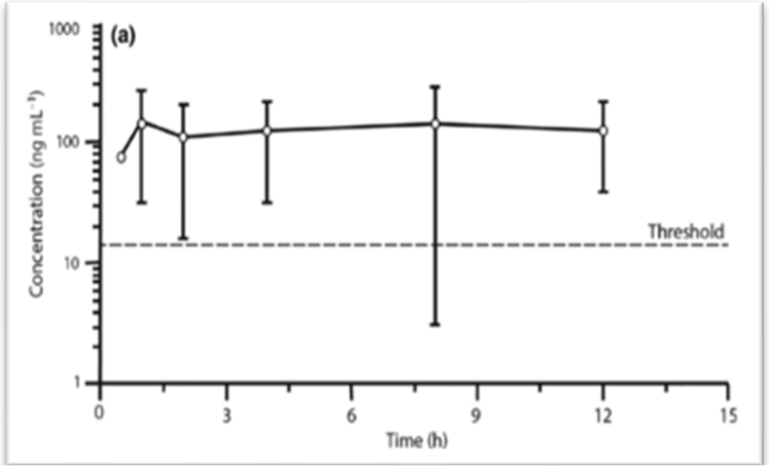
- Not fully characterized
- One published full genome sequence of *O. ophidiicola*
- Phylogenetic analysis of *O. ophidiicola* isolates
- Difference in growth rate between isolates
- New work suggests at least 6 new clades



Treatment Options

- Treatment of fungal diseases in reptiles historically problematic
- Previously treatment of ophidiomycosis with voriconazole and itraconazole
- Terbinafine concentrations >15 ng/mL effective against *O. ophiodiicola in vitro*
- Pharmacokinetic study in healthy snakes
  - Nebulization with 2 mg/mL terbinafine solution
  - Therapeutic plasma levels achieved
  - No side effects observed
- Advantages of nebulization



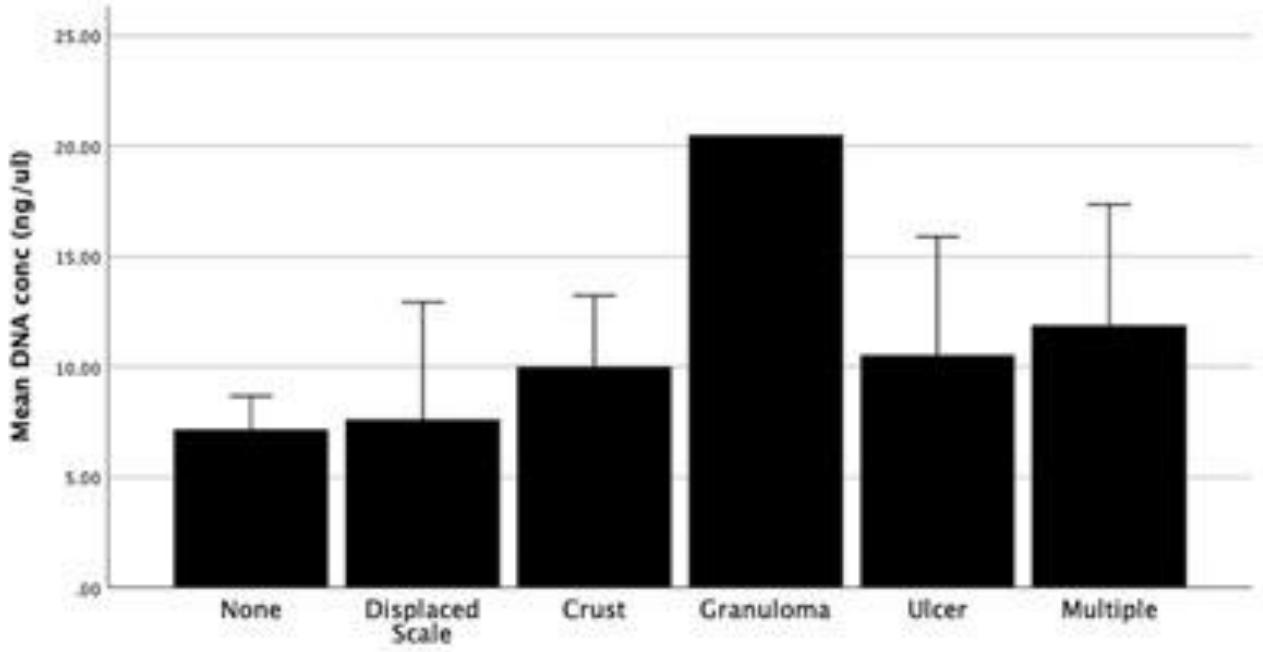


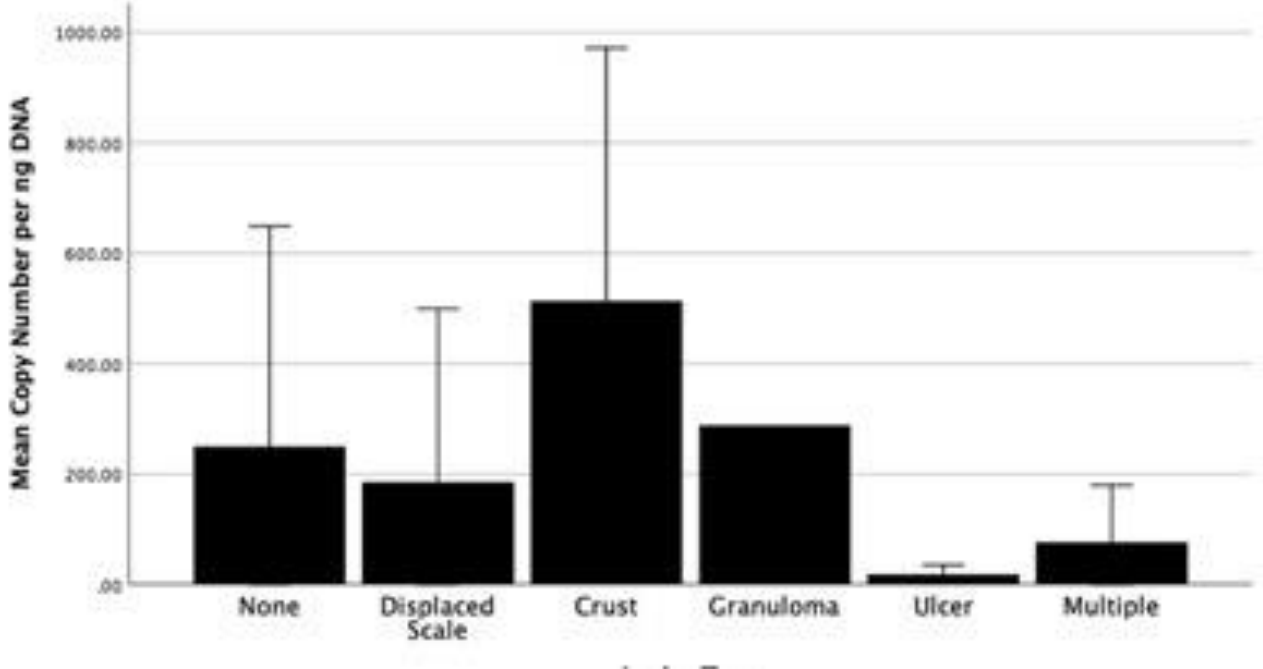
Disinfectants

Disinfectant	1:20	1:65	1:110	1:155	1:200
3% Bleach	0	0	0	0	0
10% Bleach	0	0	0	0	0
70% Ethanol	0	0	0	0	0
NPD	0	0	0	0	0
Benzalkonium chloride	0	0	0	0	0
Lysol Power Bathroom Cleaner	0	0	0	0	0
Lysol All Purpose Cleaner	0	0	0	0	0
CLR Bath & Kitchen Cleaner	0	0	0	0	0
409	0	0	0	0	0
Simple Green All-Purpose Cleaner	34	25	15	8	5
2% Nolvasan	90	49	21	16	6
Spectracide Immunox High	TNTC	444	232	173	120
Spectracide Immunox Low	625	537	319	225	112
Saline	TNTC	183	134	95	64

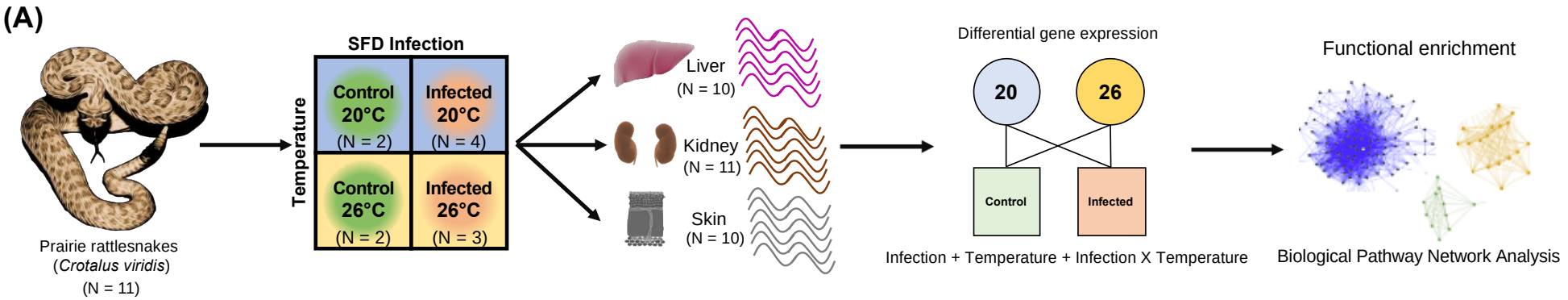
Challenge Study

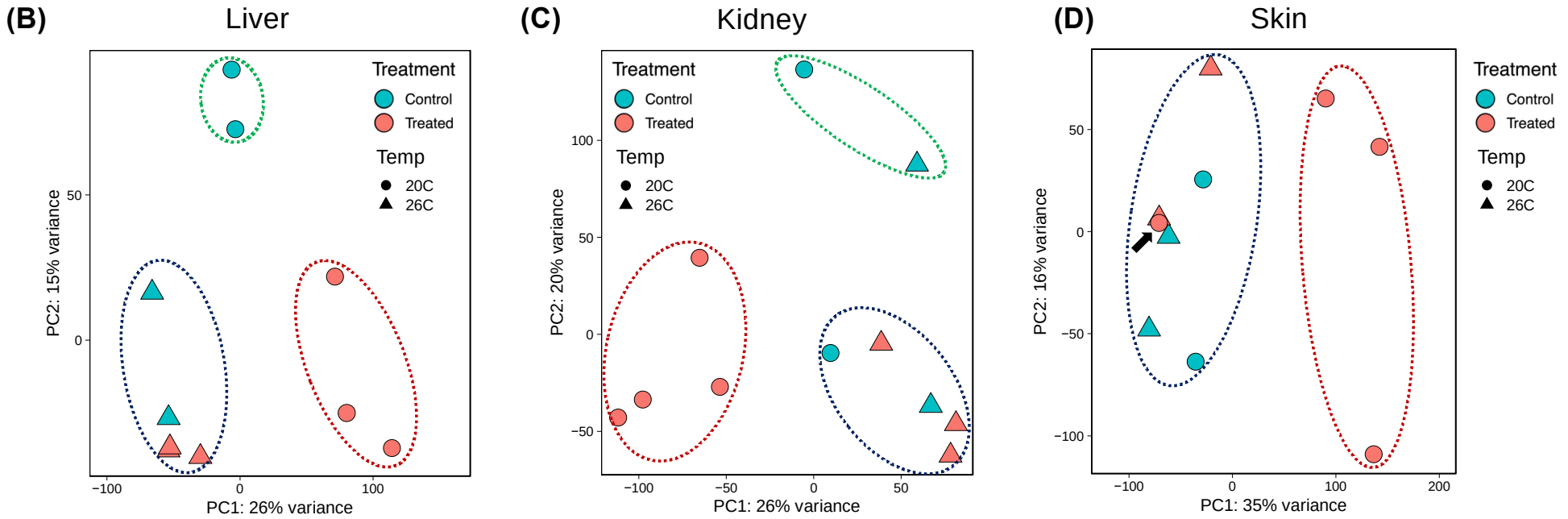
- Median Survival Time
  - 69 days at 20C
  - 62 days at 26C
- Animals at warmer temperatures shed 2-3x more frequently
- Animals had higher temperature were more active
- Crust most common lesion in low temp group, ulcer most common in high temp group

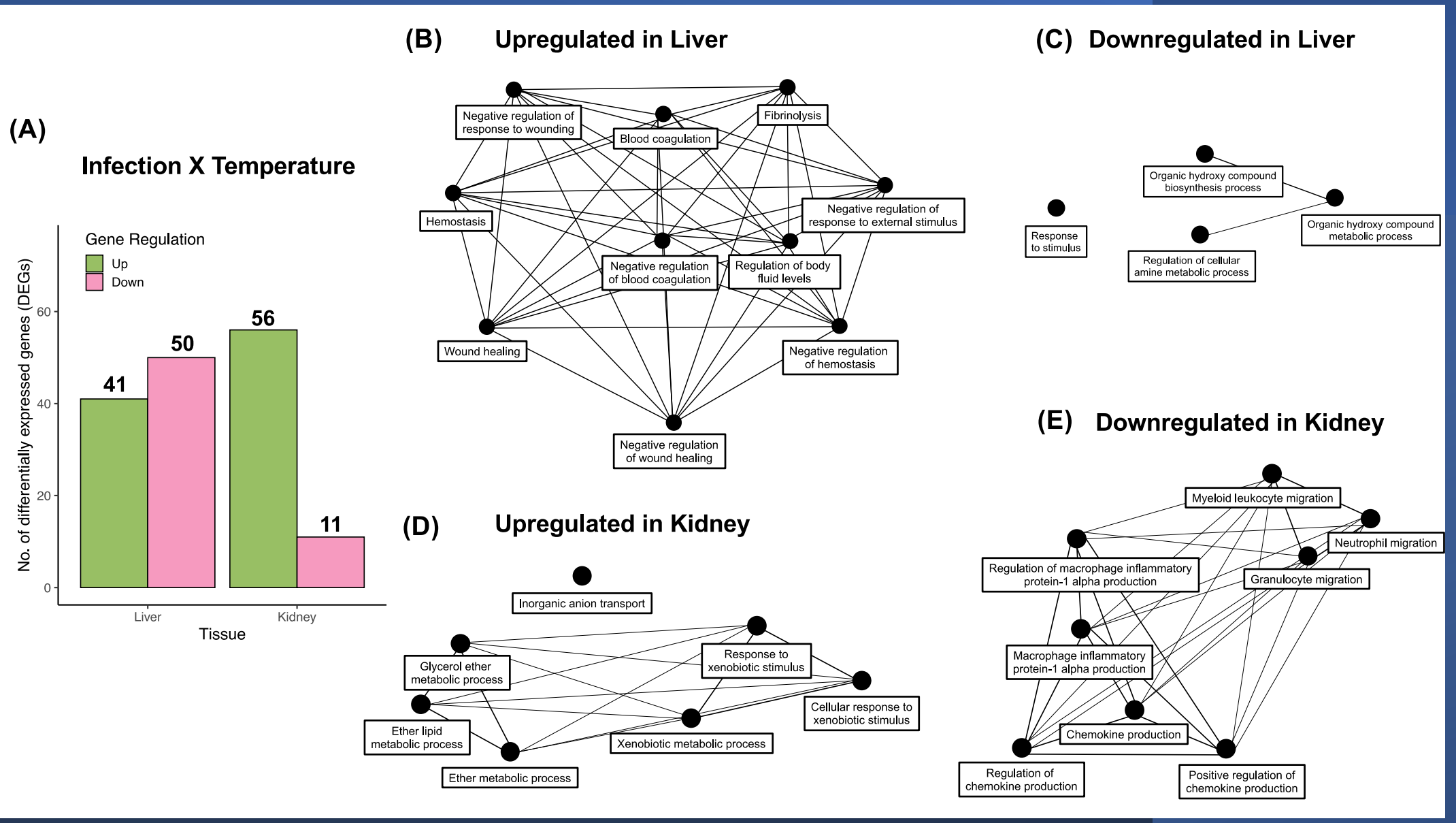




Challenge Study






(A)
Infection X Temperature
Gene Regulation
Up
Down
No. of differentially expressed genes (DEGs)
0
20
40
60
41
50
56
11
Liver
Kidney
Tissue
(B)
Upregulated in Liver
Negative regulation of response to wounding
Fibrinolysis
Blood coagulation
Hemostasis
Negative regulation of response to external stimulus
Negative regulation of blood coagulation
Regulation of body fluid levels
Wound healing
Negative regulation of hemostasis
Negative regulation of wound healing
(C)
Downregulated in Liver
Organic hydroxy compound biosynthesis process
Response to stimulus
Organic hydroxy compound metabolic process
Regulation of cellular amine metabolic process
(D)
Upregulated in Kidney
Inorganic anion transport
Response to xenobiotic stimulus
Glycerol ether metabolic process
Cellular response to xenobiotic stimulus
Ether lipid metabolic process
Xenobiotic metabolic process
Ether metabolic process
(E)
Downregulated in Kidney
Myeloid leukocyte migration
Neutrophil migration
Regulation of macrophage inflammatory protein-1 alpha production
Granulocyte migration
Macrophage inflammatory protein-1 alpha production
Chemokine production
Regulation of chemokine production
Positive regulation of chemokine production

Snakes on DoD Lands

- There are more snake species on DoD lands than any other herpetofaunal group
- 131 snake species confirmed present on continental U.S. military lands (85% of all snakes species in the U.S.)

Snakes on DoD Lands

- There are 27 species of venomous snakes confirmed present on military lands in the continental U.S.
- Approximately half of the military sites with Integrated Natural Resource Management Plans have at least one venomous snake species confirmed present


J.D. Willson


Chris Petersen


Paul Block

Snakes on DoD Lands

- The venomous Eastern Copperhead is found on more military sites (84) than any other venomous snake species
- The most common non-venomous snake on military lands is the North American Racer, which is occurs on approximately 200 military sites


Seth Berry


Chris Petersen

Snakes on DoD Lands

- The Eastern Indigo Snake, Eastern Massasauga, Brown Gartersnake, Louisiana Pinesnake, and Black Pinesnake are federally protected by the Endangered Species Act and confirmed present on DoD lands.
- At-risk snake species confirmed present on DoD lands, including the Desert Massasauga, Eastern Diamond-backed Rattlesnake, and Florida Pinesnake


Dirk Stevenson


Jeffery G. Davis


Chris Melder

Department of Defense
Snake Fungal Disease Survey: Natural Resource Manager Training and Data Collection
Part II

Research Goals and Objectives

- Sampling from 2021-2022, continued from 2018
- Close information gaps in the scientific understanding of this disease
  - Spatial distribution
  - Species affected
  - Environmental conditions
- Raise awareness and train/educate personnel on military installations nationwide
- Prevention of negative impacts to military readiness as a result of degrading ecosystem health

Methods

- We provided a standardized field datasheet, sampling protocol, and biosecurity procedures
- We provided swabbing kits (swabs, sample tubes, etc.)
- We provided an online training video
  - **DoD Training Video 2018 - YouTube**
- Participants sent resulting field samples to University of Illinois for analysis
- We provided a summary report and installation-specific report of results

Field Sampling

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)**

Results

- Kits from 47 installations returned results
- 1900 swabs from 826 individuals representing 81 species/subspecies in 31 states were observed and tested for Ophidiomyces
- 36 species were detected with *O. ophidiicola*

qPCR Results

1) Negative (no clinical signs or qPCR detection of *O. ophidiicola* DNA; **586** (462 in 2018) individuals)

2) *Ophidiomyces* present (qPCR detection in absence of clinical signs; **74** (64 in 2018) individuals)

3) Possible ophidiomycosis (presence of clinical signs in absence of qPCR detection; **72** (82 in 2018) individuals)

4) Apparent ophidiomycosis (presence of clinical signs and qPCR detection; **84** (49 in 2018) individuals)

Categories based on Baker et al. 2019

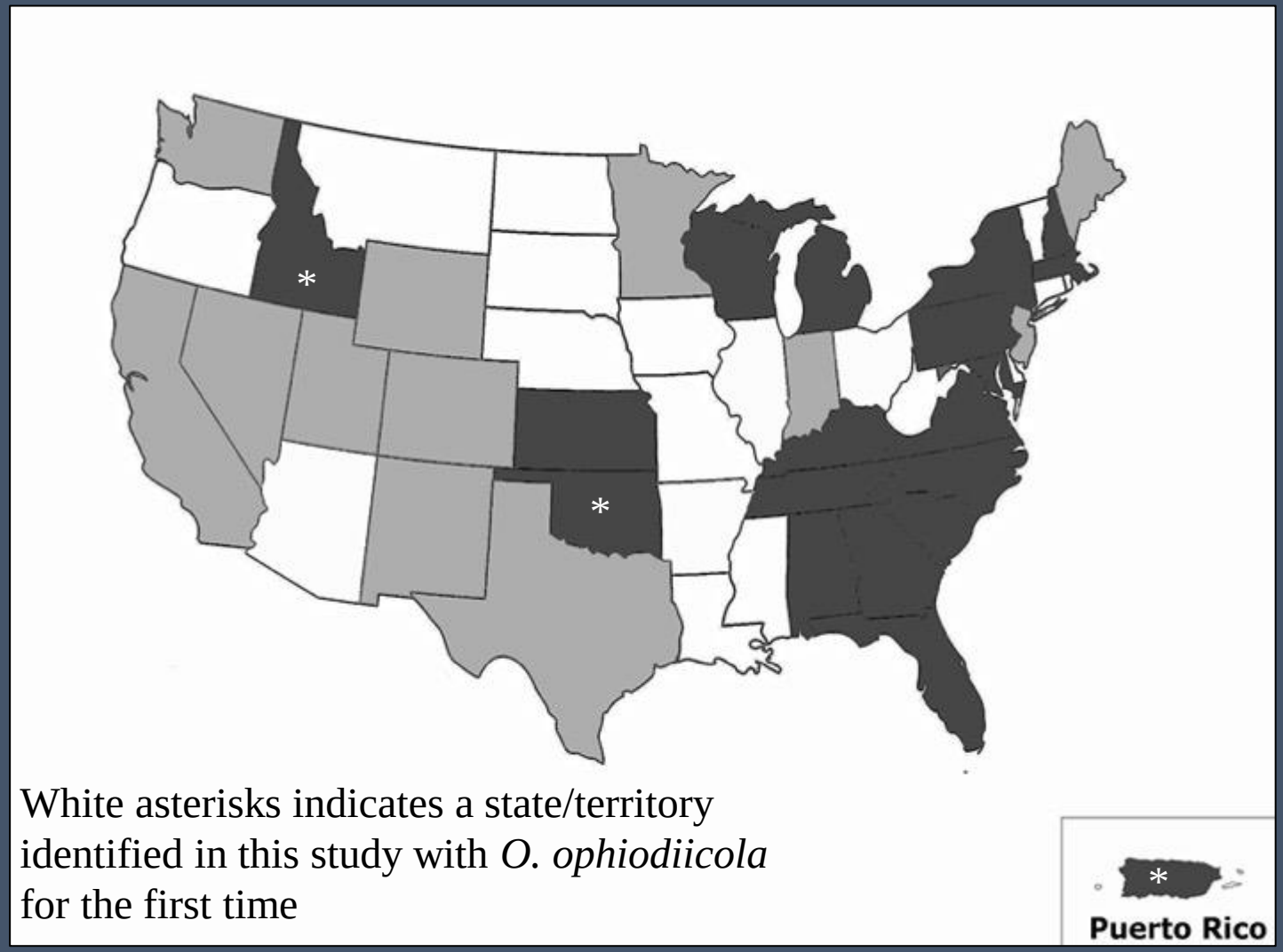
Results

- *Nerodia sipedon* had 2.83 times higher Odd's of being detected than other species
- *Drymarchon* and *Coluber constrictor* more likely to exhibit signs when positive
- Nerodia less likely to exhibit clinical signs when positive
- We report the first detections of *O. ophidiicola* in NM, previously ID, OK, and Puerto Rico in 2018

Spatial Distribution of *O. ophiodiicola* Detection in Snakes on Military Installations Sampled in 2018

Map Legend

White = states not sampled
Light Grey = states with no detection of *O. ophiodiicola*,
Dark Grey = states detected with *O. ophiodiicola*

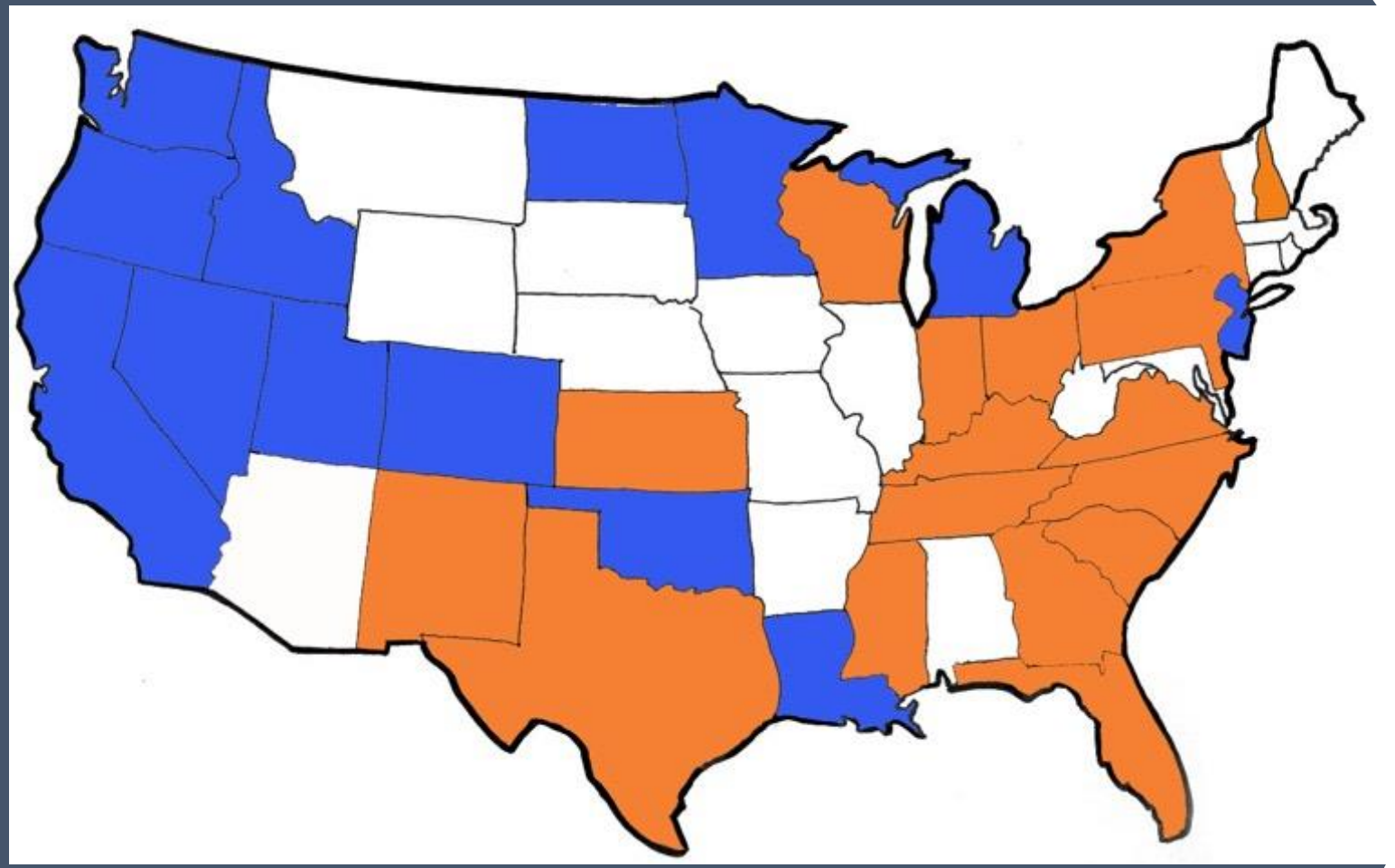


White asterisks indicates a state/territory identified in this study with *O. ophiodiicola* for the first time

Spatial Distribution of *O. ophiodiicola* Detection in Snakes on Military Installations Sampled in 2020-21

Map Legend

White = states not sampled
Blue = states with no detection of *O. ophidiicola*,
Orange = states detected with *O. ophidiicola*



Summary

- Greater prevalence to previous study (19% versus 17%)
- Widespread on the landscape of military lands
- All 36 species identified have previously been documented in other studies
- High prevalence species have consistently high prevalence throughout the literature, causes of disease still unknown
- New Mexico had first positive free-ranging snake
- Military readiness may be impacted by at-risk species protections

Questions?