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Project Title: Early Detection of *P. destructans* in New Mexico

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Progress Report March 1 through June 23, 2018

Summary and Significance of Project:

Early detection of *Pseudogymnoascus destructans* (a.k.a. *Pd*) is an important management tool in reducing the spread of this white-nose syndrome (WNS) causing fungus. With WNS in two neighboring states, New Mexico is now on the leading edge of the spread of WNS. This study aims to monitor caves across the state of New Mexico in Spring 2018 and Fall 2018, in order to determine if *P. destructans* has entered New Mexico. Using real-time PCR methods, this study will focus on caves/mines that are known hibernacula for either *Corynorhinus townsendii* (COTO) or *Myotis* spp. bats, in order to determine if *P. destructans* is present in any of the caves/mines selected as among the most vulnerable to *P. destructans* infection.

Summary of Methods:

Caves and mines were selected in consultation with New Mexico Department of Game and Fish, the Bureau of Land Management, the National Park Service, and NM Abandoned Mine Land Program. Permits and Section 106 compliance letters were obtained from the appropriate (i.e., land management) agency for each cave/mine. A total of 17 caves and one mine were sampled in the spring of 2018 from March through May (Table 1). Due to the urgency of early detection in NM, we chose to sample all of the identified caves in the spring, and will retest a subset of caves in the fall.

The sample sites within the caves were selected based on presence of bats (fresh guano in area) or pinch points in caves where both humans and bats would have to pass. If guano was present, then it was collected. Figure 1 shows examples of samples and sampling sites. At each sample site, guano and/or soil were collected using sterile techniques. Approximately 10-30 cc of guano and/or soil were sterilely collected into a sterile 50 cc falcon tube. This tube was designated the primary sample. The sample was thoroughly mixed through agitation and then divided into two additional sterile tubes. The primary sample and one replicate were preserved with sucrose lysis buffer (SLB), which breaks open microbial cells and stabilizes the DNA for long-term preservation. The third tube was left as is with no additional preservative. All tubes were placed on ice/stored at 4°C as soon as possible and stored at -80 °C upon returning to the lab. Additionally, at a few locations, swabs of dead bats or other isolated guano samples were collected. In BLM caves 45 and 55 and Cottonwood cave, dead bats were collected in sterile whirl packs. These bat samples did not show signs of suspicious fungus, but will be tested if the soil/guano samples in the area are positive for *P. destructans*. A total of 24 people, including several volunteers, helped to collect the samples in this project.

Table 1: Summary of Caves, Samples Sites and Samples.

Cave	County	Number of Samples Sites	Number of Primary samples	Number of Additional Samples (swabs etc.)	Number of Samples Extracted	Number of Samples positive for *Pseudogymnoascus* spp.	Number of Additional Samples Possibly Positive for *Pseudogymnoascus* spp
Pinon	Chavez	3	3		3	0	2
A'a	Cibola	3	3		3	1	1
AJ	Cibola	3	3		3	3	0
Bat	Cibola	3	3		3	2	0
Brewers	Cibola	3	3		3	3	0
Four Windows	Cibola	3	3		3	0	1
Hummingbird	Cibola	4	4		4	2	1
Junction	Cibola	3	3		3	1	0
West	Cibola	2	2		2	1	0
BLM CAVE 55	DeBaca	3	3		3	2	0
Carlsbad Cavern Bat Cave	Eddy	6	6		6	1	4
Carlsbad Cavern LC	Eddy	2	2	1	3	1	0
Carlsbad Cavern Right Hand Fork	Eddy	2	2		2	0	2
Cottonwood	Eddy	4	4		4	3	0
Goat	Eddy	4	4		4	1	2
Lake	Eddy	4	4	1	5	0	0
Ogle	Eddy	5	5		5	0	0
Fort Stanton	Lincoln	6	6		6	4	0
BLM Cave 45	Lincoln	5	5	5	10	4	0
Nancy Mine	Socorro	4	4		4	1	0
		Total	72	7	79	30	13


A
B
C
CC 180317-23
Site 3 ~
Upper Lake


D
E
F
G
Bat Cave Site S

Figure 1: Examples of sampling site, methods, and samples. A. Northup and Hathaway sampling in Fort Stanton Cave. Personal protective equipment such as respirators and gloves were worn while sampling and Hathaway, Strach, and Northup have been certified on respirators by University of New Mexico Safety personnel. Photo by D. Buecher. B. Eddie Strach helping to sample in Goat Cave. The photo shows the area sampled, which was several square feet in size. Photo by Kenneth Ingham. C. Large guano pile that was sampled from Carlsbad Cavern. Note the fungus (white) on the guano. Photo by Diana Northup. D. Hathaway scooping individual guano pellets into a sterile falcon tube. Photo by Diana Northup. E. Example of guano with fungus on surface. Photo by Diana Northup. F. Example of sampling site, showing that a single sample could come from several adjacent areas in a cave. Photo by Kenneth Ingham G. Sample that was a mixture of guano and soil/sediment. Soil collected if there was not a lot of guano present in area. Photo by Jennifer Hathaway.

The primary sample was extracted in duplicate using the Qiagen Power Soil Kit following the manufacturer's protocol, with the following modifications: samples were bead beaten for 1.5 minutes at medium speed after the addition of solution C1. Approximately 0.25 g of the sample was used in each extraction and were eluted in 50 *ul* of solution C6. A negative control extraction was also performed to ensure there was no contamination of reagents. This control was exposed to the same condition and reagents as the samples, but with no sample added.

In order to determine if the extraction was performed cleanly and that fungus was present in the sample, a PCR was performed using universal fungal primers ITS1F and ITS4. This allows for detection of any kind of fungus in the sample. A PCR was also performed on the negative control extraction as quality control of the extraction. If the PCR of the negative control extraction had no band, then we can assume that the extraction was done without contamination.

All samples were then PCR-tested with primers designed to test for the presence of *Pseudogymnoascus* spp*.,* including *Pd* (Lorch, et al. 2010). The aim of this step is to identify samples with *Pseudogymnoascus* spp*.* present in them that warrant testing with qPCR. qPCR is a more expensive technique, thus use of the Lorch et al. (2010) primers allows us to target our testing more effectively.

qPRC following the method in Shuey et al 2014 is currently being performed on the samples that tested positive for *Pseudogymnoascus* spp*.*

Summary of Progress:

All of the 72 primary samples that were collected have had their DNA extracted. All but one sample was positive for fungal DNA. Seven additional samples were extracted, which consisted of swabs of dead bats from BLM cave 45, or addition guano and soil samples from each of two caves in the Carlsbad system. A total of 79 samples were tested with the *Pseudogymnoascus* spp. primers and 30 of these samples were positive for *Pseudogymnoascus* spp. An additional 13 samples had bands of the wrong size using these primers. These samples will be also tested with qPCR. qPCR testing is currently underway on these 43 samples.

Timeline:
qPCR testing is expected to be complete by August 31, 2018. A report to the agencies involved, detailing the results of samples from the caves/mines on the land that they manage, will be completed as soon as the qPCR testing is finished. Should some of the samples be positive for *P. destructans*, we propose to ask Dr. Jeff Foster of Northern Arizona University, and a co-author on the Shuey et al. (2014) paper, to do a confirmatory test before the results are released. A complete report summarizing all the results will be submitted to Game and Fish.

The remaining ~ 21 samples will be collected in August to October 2018, and the methods described above will be performed on those samples.

References:

Lorch, J.M., Gargas, A., Meteyer, C.U., Berlowski-Zier, B.M., Green, D.E., Shearn-Bochsler, V., Thomas, N.J. and Blehert, D.S., 2010. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation*, *22*(2), pp.224-230.

Shuey, M.M., Drees, K.P., Lindner, D.L., Keim, P. and Foster, J.T., 2014. Highly sensitive quantitative PCR for the detection and differentiation of *Pseudogymnoascus destructans* and other *Pseudogymnoascus* species. *Applied and environmental microbiology*, *80*(5), pp.1726-1731.