AN ABSTRACT OF THE THESIS OF

Xi Chen for the Master of Science

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Title: Diversity of the Type I Intron/ITS Region of the 18S rRNA Gene in *Geomyces* species from the Red Hills of Kansas

Abstract approved:

White-nose syndrome (WNS) is an emerging wildlife disease that has caused the most rapid wildlife population declines ever reported and is threatening all temperate bat species. Analysis of the growth from WNS inflicted bats has conclusively identified the causative agent of WNS, a fungus designated *Geomyces destructans*. Since the first report from New York in 2006, WNS has been detected in 16 additional states and implicated in over five million bat deaths in North America. The Gypsum caves found throughout the Red Hills of Kansas have the state's most diverse and largest population of cave roosting bats, and are home to some Tier 1 ranking species as noted by the Kansas Comprehensive Wildlife Conservation Plan (KCWCP). Currently, WNS has not been detected in the Gypsum caves. However, the rapid westward movement of WNS from the Eastern United States, the likely occurrence of WNS in neighboring counties of Oklahoma, and the already fragile populations of bats in the Red Hills of Kansas dictate aggressive action to help aid the understanding of this impending epizootic disease. In this study, cave soil was obtained from the Red Hills. Using the polymerase chain

reaction, a 624 nucleotide DNA fragment specific to the Type1 Intron/ITS region of the 18S rRNA gene from *Geomyces* species was amplified. Subsequent DNA sequencing and direct comparison to the same genetic locus in *G. destructans* was performed. The data indicates that *G. destructans* DNA was detected, along with 26 *Geomyces* variants. Continued surveillance to monitor trends of *G. destructans* distribution in the Red Hills of Kansas is critical to the management of a possible WNS outbreak should it occur.

DIVERSITY OF THE TYPE I INTRON/ITS REGION OF THE 18S rRNA GENE IN *GEOMYCES* SPECIES FROM THE RED HILLS OF KANSAS

A Thesis

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by

Xi Chen

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Approved by Major Advisor

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Approved by Committee Member

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Approved by Committee Member

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Approved for Department of Biological Sciences

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Approved for Dean of Graduate Studies and Research

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PREFACE

This thesis was prepared following the publication style of the American Society for Microbiology.

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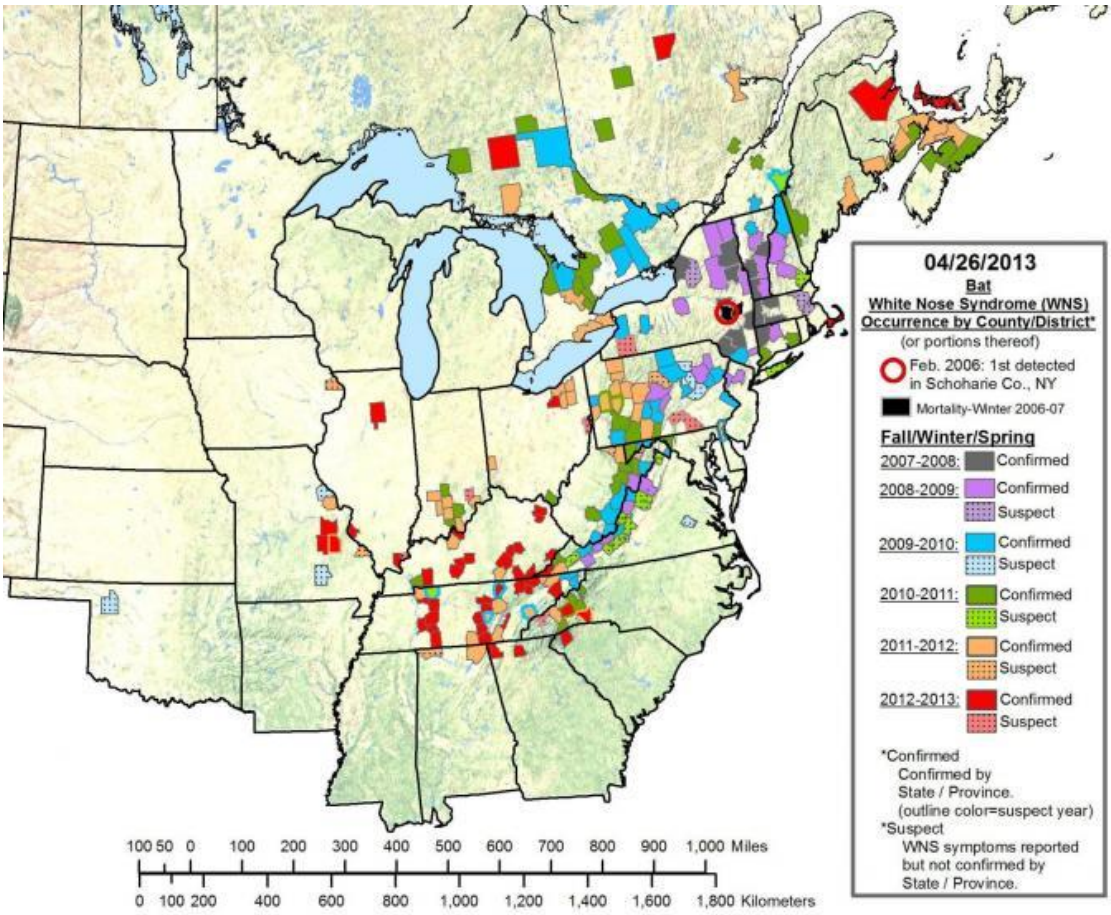
Introduction

White-nose syndrome (WNS) is an emerging wildlife disease that has caused the most rapid wildlife population declines ever reported and is threatening all temperate bat species (10, 25). WNS is characterized by visually apparent white fungal growth on exposed skin of ears, muzzle, tail, snout, and wing membranes of hibernating bats (4, 19). The fungus, designated as *Geomyces destructans*, has been conclusively identified as the causative agent of WNS (19, 27). This fungus causes bats to lose fat reserves needed to survive winter hibernation and causes them to prematurely emerge from hibernacula in mid-winter (3, 6).

Since its initial detection on 16 February 2006 at Howes Cave, Albany, NY, WNS has resulted in the death of more than five million bats in North America (10). This far exceeds the magnitude of any previously known mortality events in bats (3, 6). *Geomyces destructans* has been found in nine species of bats in North America, and population models even predict that little brown bats (*Myotis lucifugus*) will face regional extinction in the near future, which could cause a serious imbalance in the ecosystem with unforeseen consequences (4, 10). It is assumed that all the species of cave hibernating bats will be at risk as WNS spreads to new areas (6). Starting from New York, WNS has spread rapidly throughout seventeen U.S. states and four Canadian provinces (Figure 1). It is moving westward, as evidenced by its detection in Missouri and Oklahoma.

The Gypsum caves found throughout the Red Hills of Kansas have the state's most



Map by: Cal Butchkoski, PA Game Commission

Figure 1. A map of WNS in North America. (www.tnbwg.org; March, 2013)

diverse and largest population of cave roosting bats (Figure 2). Currently, WNS has not been detected in the Gypsum caves. However, the rapid westward movement of WNS from the Eastern United States and the already fragile populations of bats in the Red Hills of Kansas dictate the scientific community must act aggressively to help aid the understanding of this impending epizootic disease.

To further investigate the possible occurrence of WNS in the Red Hills caves, a bat survey was conducted during the winter months of 2011-2012 (Appendix 1). Even though no evidence of WNS was detected, soil samples were collected to determine if *G. destructans* was present. Previously, Lorch et al. (16) developed a PCR-based diagnostic approach to detect *G. destructans* based on the amplification of the Type1 Intron/ITS region of the 18S rRNA gene. This technique was subsequently employed by Lindner et al. (15) to detect *G. destructans* in bat hibernacula. Based on this established methodology, the goal of this study was to conduct a PCR-based survey of the Red Hills caves to detect the occurrence of *G. destructans*, parallel to the studies of Lindner et al. (15).

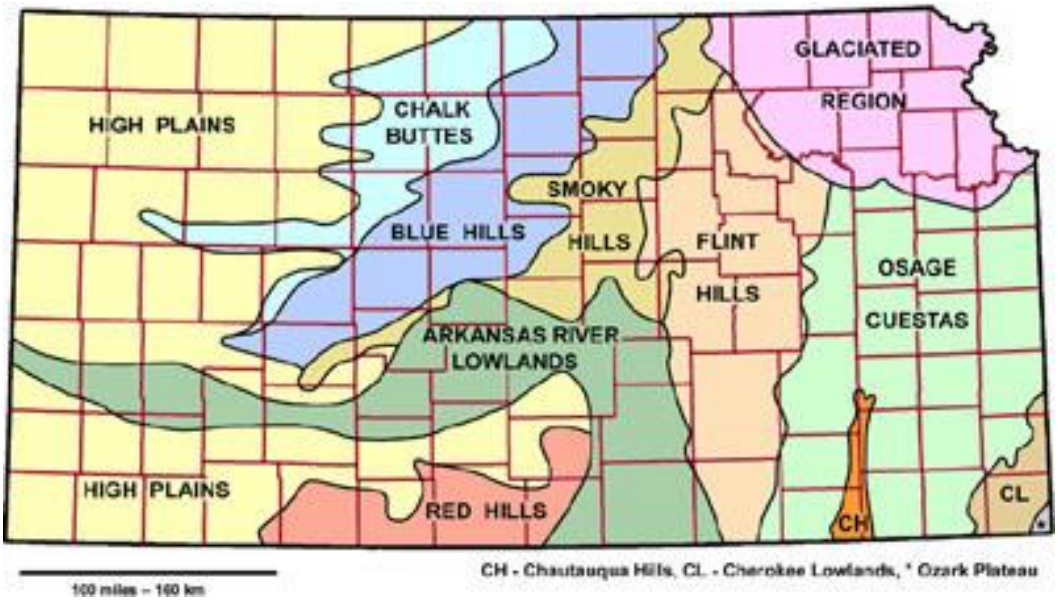

GLACIATED
REGION
HIGH PLAINS
CHALK
BUTTES
SMOKY
HILLS
BLUE HILLS
FLINT
HILLS
OSAGE
CUESTAS
ARKANSAS RIVER
LOWLANDS
HIGH PLAINS
RED HILLS
CH
CL
100 miles = 160 km
CH - Chautauqua Hills, CL - Cherokee Lowlands, * Ozark Plateau

Figure 2. Map of Kansas.

Materials and Methods

Sample Collection

Soil samples used in this study were collected from caves located in the Red Hills of Kansas by Dr. William Jensen (Emporia State University) and his research team from December 2011-January 2012. Using sterile spatulas, soil samples were placed into sterile collection bags. In total, 12 samples were obtained from each cave (n=16). All samples were stored at 4 °C until used for DNA purification.

Bacterial Strains and Media Used in This Study

Escherichia coli TG1 was used as the host strain for transformation experiments. It was routinely propagated at 37 °C in Luria-Bertani (LB) media (10 g Bactotryptone, 5 g yeast extract, 10 g NaCl/L). Agar plates were prepared by adding agar (20 g/L) to liquid media. Ampicillin was used at 100 μg/ml.

DNA Isolation

Total DNA was isolated from soil samples using a ZR Soil Microbe DNA MicroPrep™ (Zymo Research; Irvine, CA) according to the manufacturer's instructions. Plasmid DNA from recombinant *E. coli* TG1 was isolated using a QIA prep® Spin Miniprep Kit (Qiagen; Valencia, CA) according to the manufacturer's instructions. All DNA was stored at 4 °C until used.

DNA Concentration Determination

DNA concentration was determined using a Nanodrop 2000c spectrophotometer (Thermo Scientific™; St. Louis, MO). The final concentration of all DNA samples was adjusted to 50 µg/ml using H_2O.

Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) was used to amplify gene specific regions from DNA templates. The amplification process was carried out as described in Table 1 using a Bio-Rad T100 thermocycler (Bio-Rad; Hercules, CA). Reactions typically consisted of a deoxynucleotide triphosphate mix (200 µM each dNTP), polymerase specific reaction buffer, 50 ng DNA, 1.5 mM $MgCl_2$ and 1 U *Taq* polymerase for the 16S rRNA amplification and screening for the 624 bp Type1 Intron/ ITS region of the 18S rRNA gene. Once an amplicon was obtained, PCR was repeated using 1U of High-Fidelity DNA Phusion Polymerase (Thermo Scientific™) to obtain the fragment for subcloning.

Agarose Gel Electrophoresis

To effectively separate DNA fragments of various sizes, agarose gel electrophoresis was employed according to standard conditions (21). Briefly, 30 ml of 1X TAE buffer prepared from a 50X TAE stock (242 g Tris, 57.1 ml acetic acid, and 4 ml 0.5 M EDTA in 1 L of H_2O), 0.3 g of agarose, and 2 µl of 10 mg/ml ethidium bromide (EtBr) were mixed

Table 1. PCR primers and amplification conditions.

Primer Sequence	Reaction Conditions[a]	Reference	Note
CC[F] ---5'- CCA GAC TCC TAC GGG AGG CAG C- 3'	94 °C/5min	(20)	Amplification of the 16S rRNA gene
CD[R] --- 5'- CTT GTG CGG GCC CCC GTC AAT TC- 3'	94 °C/1min 55 °C/1min 72 °C/45sec Repeat cycle, 29X 72 °C/ 5min 12 °C/∞		
Gd enrichment [F] --- 5'-GGG GAC GTC CTA AAG CCT- 3'	94 °C/5min	(16)	Amplification of the 624bp Type 1 Intron/ITS Region of the 18S rRNA gene of *Geomyces* species
Gd enrichment[R] --- 5'-TTG TAA TGA CGC TCG GAC- 3'	94 °C/1min 52 °C/1min 72 °C/1min Repeat cycle, 29X 72 °C/ 5min 12 °C/ ∞		

[a]Reaction conditions described by Lorch et al., 2010 were slightly modified in this study.

and heated in the microwave until the agarose dissolved. The mixture was poured into a gel casting tray containing a gel comb. After solidification, the comb was removed and 1X TAE buffer was added to completely cover the gel. Subsequently, DNA samples were mixed with loading buffer, loaded into the wells, and electrophoresed using a Bio-Rad model 250/2.5 power supply (Bio-Rad), at ~100 volts for 30-40 min. DNA was visualized after electrophoresis using a UV Intensity Transilluminator (Fisher Scientific™; St. Louis, MO).

DNA Purification from an Agarose Gel

DNA was excised from agarose gels using a razor blade. The slice of agarose containing the DNA was placed in a 1.5 ml microcentrifuge tube and purification accomplished using a Zymoclean™ Gel DNA Recovery Kit (Zymo Research) according to the manufacturer's instructions.

DNA Ligation

DNA was ligated into pJET 1.2 (Thermo Scientific) using a Fast-Link™ DNA ligation kit (Fisher Scientific) according to the manufacturer's instructions. Reactions consisted of 1.5 µl 10X Fast-Link Ligation Buffer, 0.75 µl of 10 mM ATP, 1 µl of linearized pJET1.2, 1 µl of insert DNA, 2 U of DNA ligase, and 9.75 µl of distilled water to reach a total volume of 15 µl. Reaction mixtures were incubated at room temperature for 15 min.

Preparation of Competent Cells

Escherichia coli TG1 was grown in 2X LB medium at 30 °C overnight with shaking at 250 rpm. Subsequently, 0.5 ml of an overnight culture was used to inoculate 200 ml of fresh 2X LB in a flask at 30 °C with shaking. When the OD_{600} of the culture reached 0.3, 4 ml of 1M $MgCl_2$ was added and incubation continued until an OD_{600} of 0.45-0.55 was obtained. Subsequently, the culture was placed on ice for 2 hrs prior to centrifugation at 3000 rpm at 4 °C for 5 min in a J2-HS centrifuge (Beckham Coulter Inc; San Diego, CA). The cell pellet was resuspended in ice-cold 100 mM $CaCl_2$ media (0.05 M $CaCl_2$, 0.04 M $MnCl_2$, and 0.02 M CH_3COON, pH=7.5). After incubation on ice for 40 min, cells were pelleted by centrifugation and resuspended in fresh ice-cold 100 mM $CaCl_2$ media containing 15% glycerol. The competent cells were stored in 100 µl aliquots at -80 °C.

Transformation

Ligation reactions were transformed into *E .coli* TG1 competent cells by mixing 5 µl of each ligation mixture with 100 µl of cells. After incubation on ice for 15 min, reactions were heat shocked at 42 °C for 90 sec. After a 1 min recovery on ice, 900 µl of LB medium was added and the transformation mixture incubated at 37 °C for 1 hr. Subsequently, 100 µl of the transformation mixture was spread onto LB/ampicillin (100 µg/ml) plates followed by incubation at 37 °C overnight. Recombinants were selected for further analysis.

DNA sequencing

DNA sequencing was carried out by the DNA Sequencing Facility at the University of Arkansas for Medical Science, Little Rock, Arkansas.

BLAST analysis

The Basic Local Alignment Search Tool (BLAST) (1) was performed to compare DNA sequences of cloned samples to the 624 bp *G. destructans* Type 1 Intron/ ITS Region of the 18S rRNA gene (GenBank: EU884921.1).

Results

Total DNA was isolated from all 189 soil samples and standardized to a concentration of 50 μg/ml. Amplification of 16S rRNA gene from each DNA sample was successfully performed, indicating the DNA was of sufficient quality for PCR (Figure 3). Using *Geomyces* specific primers for the 624 bp Type 1 Intron/ITS Region of the 18S rRNA gene, the expected amplicon of 624 bp was identified in 12 samples from 6 different caves (Figure 4). A heterogeneous 624 bp fragment was eluted from the agarose gel representing each positive cave and cloned into pJET1.2. A total of 302 recombinant clones (~50/ cave) were selected and subjected to DNA sequencing. BLAST analysis demonstrated the 624 bp *G. destructans* sequence could be identified in soil samples from 4 caves along with 26 different *Geomyces* variants (Table 2). Appendix 1 contains the complete 624 bp DNA sequence for *G. destructans* and all variants described in this study.

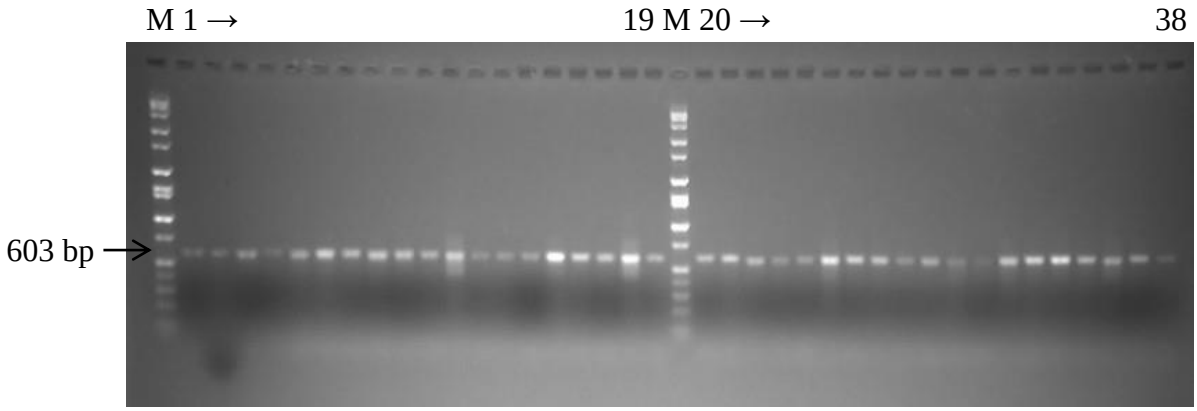

M 1 →
19 M 20 →
38
603 bp

Figure 3. Amplification of 16S rRNA gene. This is a representative agarose gel of all 189 samples. Lane M – DNA ladders; Lane 1-38 – PCR amplification of the 603 bp 16S rRNA gene.

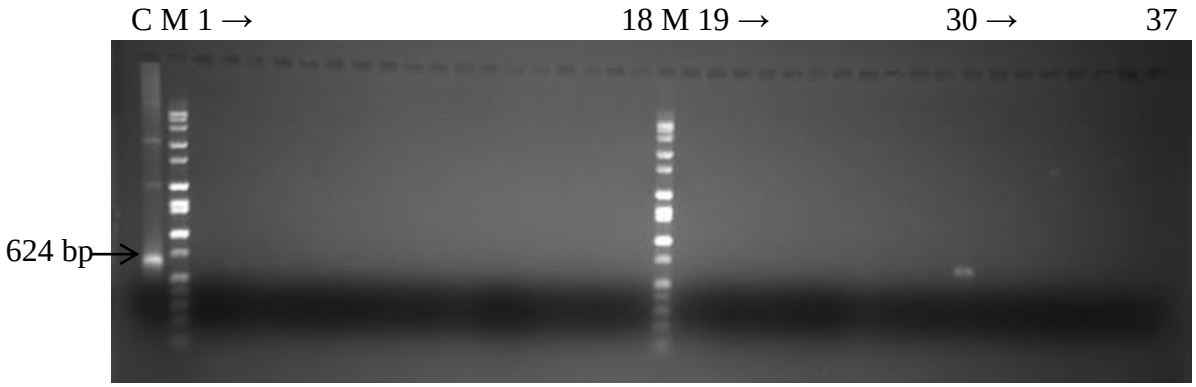

C M 1 →
18 M 19 →
30 →
37
624 bp

Figure 4. Amplification of the Type1/ ITS region of the 18S rRNA gene from the genus *Geomyces*. This is a representative agarose gel of all 189 samples. Lane C – 624 bp amplicon from *Geomyces destructans*; Lane M – DNA ladders; Lane 1-29 and 31-37 – negative amplifications from soil samples; Lane 30 – positive amplification of the 624 bp fragment.

Table 2. *Geomyces destructans* and type variants of the *Geomyces* Type 1 Intron/ITS Region of the 18S rRNA gene identified in this study.

Name	Number of Samples	Caves[a]	Closest GenBank Accession Number Match/(bp Homology)
G. destructans	n=62	CAC DCC DAC GEC	EU884921.1/(624/624)
Type1	n=178	BSC CAC DCC DAC GEC SBC	JX270511.1/(624/624)
Type2	n=2	BSC CAC	JX270511.1/(623/624)
Type3	n=2	BSC GEC	JX270511.1/(623/624)
Type4	n=7	BSC CAC DAC	JX270511.1/(623/624)
Type5	n=1	CAC	JX270511.1/(623/624)
Type6	n=1	CAC	JX270511.1/(623/624)
Type7	n=1	CAC	HM848977.1/(623/624)
Type8	n=1	CAC	JX270621.1/(618/625)
Type9	n=7	CAC DAC SBC	JX270626.1/(621/624)
Type10	n=1	CAC	JX270344.1/(623/624)
Type11	n=1	CAC	JX270511.1/(623/624)
Type12	n=1	DCC	JX270511.1/(624/625)
Type13	n=2	DCC SBC	JX270511.1/(623/624)
Type14	n=4	DAC SBC	JX270626.1/(621/624)
Type15	n=16	DAC GEC SBC	JX270626.1/(621/624)

Type16	n=2	DAC GEC	JX270344.1/(623/624)
Type17	n=1	DAC	JX270626.1/(620/624)
Type18	n=2	DAC	JX270511.1/(623/624)
Type19	n=2	DAC GEC	JX270511.1/(624/625)
Type20	n=1	SBC	JX270511.1/(623/627)
Type21	n=1	SBC	JX270626.1/(619/625)
Type22	n=1	SBC	JX270511.1/(620/624)
Type23	n=2	DAC SBC	JX270626.1/(620/624)
Type24	n=1	SBC	JX270626.1/(620/624)
Type25	n=1	SBC	JX270511.1/(623/624)
Type26	n=1	DCC	JX270511.1/(623/624)

[a] Big Surprise Cave, BSC; Can Cave, CAC; Dancers Cave, DAC; Dead Coyote Cave, DCC; Gentry Cave, GEC; Swartz Bat Cave, SBC.

Discussion

The genus *Geomyces* is composed of psychrophilic fungi which aid perennial plants in adapting to low-nutrient environments (7). They are also keratinophilic fungi which can degrade hairs and nails and have been studied for possible biodecompositional use (22). To date, nine *Geomyces* species have been identified, including *Geomyces asperulatus* (23), *Geomyces auratus* (24), *Geomyces cretaceous* (24), *Geomyces destructans* (11), *Geomyces laevis* (14), *Geomyces pannorum* (23), *Geomyces pulvereus* (13), *Geomyces sulphureus* (24) and *Geomyces vinaceus* (26). Among these species, *Geomyces pannorum* is reported to have the ability to cause skin infections in humans and animals (9, 12). The most notable member of this group, however, is *Geomyces destructans*, the causative agent of WNS.

Detection of the *Geomyces* genus in this study employed amplification of the Type1 Intron/ITS region of the 18S rRNA gene of *Geomyces*. The 18S ribosomal RNA (rRNA) genes are one of the most frequently used genes in eukaryotic phylogenetic studies because they are highly conserved throughout evolution and can be readily amplified with universal primers (18). Previous studies have shown that 18S rRNA genes are useful in detection and functional investigation of fungi (2, 8). Other highly conserved genes, such as16S rRNA genes, are also commonly amplified with universal primers in the identification of bacteria (5).

In this study, PCR amplification was accomplished following the established

protocol of Lorch et al. (16) using conserved primers to amplify the Type1 Intron/ITS region of the 18S rRNA gene. Unfortunately, the primer pair employed could not discriminate specifically *G. destructans* but rather *Geomyces* species. To separate the heterogeneous 624 bp 18S rRNA amplicon, purified DNA was cloned into the cloning vector pJET1.2. Cloning with pJET1.2 provides for a high percentage of recombinants since recircularized pJET1.2 vector will not propagate due to the production of a lethal restriction enzyme. The DNA sequence of ~50 recombinants from each putative positive cave was determined and compared to the 624 bp *G. destructans* Type1 Intron/ITS region of the 18S rRNA gene (GenBank: EU884921.1).

Results of this study confirmed the occurrence of the *G. destructans* gene in Red Hills cave soil, suggesting the possible presence of the fungus. However, no attempt was made to isolate viable *G. destructans* and no evidence of WNS-infected bats was observed in the caves (Appendix 2). Possible interpretations for the amplification of *G. destructans* specific DNA in the absence of WNS is that the fungus was once actively growing in the caves, but now only spore forms of the organism exist. It is also possible that *G. destructans* is a relatively new inhabitant in the cave. The occurrence of WNS in neighboring counties in Oklahoma provide a plausible explanation for its occurrence in the Red Hills of Kansas.

In addition to detecting the *G. destructans* Type1 Intron/ITS region of the 18S rRNA gene, twenty-six variants at this genetic locus were also detected, many of which

have not been reported in GenBank. Since 100% of all WNS cases are caused by *G. destructans*, the role of these variants in WNS, if any, remains to be determined. The most common variant identified in this study, designated as Type1, matched 100% to a previously studied *Geomyces* species (17). However, *Geomyces* variants found in this study were not cultured in laboratory, making it impossible to determine the role of the variants. According to the ecological niches occupied by *Geomyces*, the variants might be helping plants survive low-nutrient conditions and/or be involved in biodegradation.

In conclusion, this study provides the first analysis and detection of the environmental occurrence of *G. destructans* in Kansas. It also demonstrates the vast diversity of this genus based on one genetic locus. Even though WNS has not been detected in Kansas, evidence of *G. destructans* occurrence still highlights the possibility of fungal translocation and transmission by animal and human activities. Continued surveillance will be needed to monitor trends in *G. destructans* distribution in the Red Hills of Kansas.

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Appendix 1. DNA sequences of *Geomyces* variants in this study

***Geomyces destructans* (100% homology to GenBank EU884921.1)**

TTGTAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCAT
TTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTT
AACTATTATATAGTACTCAGACAGTATAGACAAACAGAGTTTAGTCCTCTGGCA
AGCGCTCGCCGGCCGGAGCCAGCAGCCCGAGGGCAGGCCTGCCAAAGCAAC
AAAGTGTAATAAACAAAGGGTGGTAGGTTACCCGGGAGGCCTTGCGGCAACC
CGGGCGACTACTGTAATGATCCTTCCGCAGGTTCACCTACGGAACGGTTTCGA
GTTCGTAGCGACTCACCCCTGCGCTTTCACGTAGGGCCCGACTATATCTTAAGC
AGAGCTAGGCTCCACCCACAACCACTTAGTCTGTGAACGTTACCCGTATAGCT
AGCGCTACTTAGGGTCTTCGCTGCGGATTATCCATAGTTCCCCAGAGGGAGTAT
CCATACACTCTTTTACCACCCCGTGGAGTTAGCACGGGCCCCCGCCTCGGTTTC
CCGGGCGGGTTGGTGTTGTAGGCTTTAGGACGTCCCC

Type1 (100% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type2 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC

GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCACGCCTGTCCGAGCGTCATTACAA

Type3 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATTGAATCTTTGAACGCACATTGCGCCCCC
TGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type4 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTTGGTGGAGCCTAGCTCTGCTTAAGATATAGTC
GGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCCG
TAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAGGC
CTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAGCC
TGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAAAC
TCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGG
ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCT
GGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type5 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTTGGTGGAGCCTAGCTCTGCTTAAGATATAGTC
GGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCCG
TAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAGGC
CTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAGCC

TGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAAAC
TCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGG
ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCT
GGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type6 (99% homology to GenBank JX270511.1 623/624)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCTCCGGGCTGCTGGCTTCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type7 (99% homology to GenBank HM848977.1)

GGGGACGTCCTAAAGCCTACAACACCAACCCGCCCGGGAAACCGAGGCGGG
GGCCCGTGCTAACTCCACGGGGTGGTAAAAGAGTGTATGGATACTCCCTCTGG
GGGACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGT
AACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCCTAAGATATA
GTCGGGCCCTACGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAAACCGT
TCCGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCA
AGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCA
GGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACTA
AACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type8 (99% homology to GenBank JX270621.1)

GGGGACGTCCTAAAGCCTACAACACCAACCCGCCCGGGAAACCGAGGCGGG
GGCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGG
GGAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGT
AACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATA
GTCGGGCCCTACGTGAAAGCGTAGGGGTGAGTCGCTACGAACTCGAAACCGT

TCCGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCACCCGGGTTGCCGCA
AGGCCTCTCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCA
GGCCTGCCTCCGGGCTGCTGGCTTCGGCCGGCGAGCGCTTGCCAGAGGACCT
AAACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACA
ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type9 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCACCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCCTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type10 (99% homology to GenBank JX270344.1)

GGGGACGTCCTAAAGCCTACAACACCAACCCGCCCGGGAAACCGAGGCGGG
GGCCCGTGCTAACTCCACGGGGTGGTAAAAGAGTGTATGGATACTCCCTCTGG
GGAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGT
AACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATA
GTCGGGCCCTACGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAAACCGT
TCCGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCA
AGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCA
GGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACTA
AACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATACGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type11 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGGTAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTA

ACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAG
TCGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type12 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type13 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTACTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type14 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG

GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCACCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCCTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type15 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGTA
ACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAG
TCGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTTGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type16 (99% homology to GenBank JX270344.1)

GGGGACGTCCTAAAGCCTACAACACCAACCCGCCCGGGAAACCGAGGCGGG
GGCCCGTGCTAACTCCACGGGGTGGTAAAAGAGTGTATGGATACTCCCTCTGG
GGAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGT
AACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATA
GTCGGGCCCTACGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAAACCGT
TCCGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCA
AGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCA
GGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACTA
AACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGTATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type17 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGCAGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCACCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCCTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTATGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type18 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCCCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type19 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCAC
CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type20 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCACAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTTCCGGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type21 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGTA
ACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAG
TCGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTTGGGCTGCTGGGTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTTTGAAGTACTATATAATAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type22 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCACCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCCTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC

CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type23 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGTA
ACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAG
TCGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTCTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTTGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type24 (99% homology to GenBank JX270626.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATACGGGTA
ACGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAG
TCGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTC
CGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGG
CCTGCCCTTGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCGGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type25 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGACTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC

GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA

Type26 (99% homology to GenBank JX270511.1)

GGGGACGTCCTAAAGCCTACAACACCAACCTACCCGGGAAACCGAGGTAGGG
GCCCGTGCTAACTACACGGGGTGGTAAAAGAATGTATGGATACTCCCTCCGGG
GAACTATGGATAATCCGCAGCGAAGACCCTAAGTAGCGCTAGCTATATGGGTAA
CGTTCACAGACTAAGTGGTTGTGGGTGGAGCCTAGCTCTGCTTAAGATATAGT
CGGGCCCTGCGTGAAAGCGTGGGGGTGAGTCGCTACGAACTCGAACCGTTCC
GTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGCAAG
GCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAG
CCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAA
ACTCTGTTTGTTTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTCAA

Appendix 2. Bat survey results[a]

Number of individuals counted per four bat species, across 17 caves or bluff crevices, in the Red Hills region of Kansas and Oklahoma in December 2011 and January 2012. For this report, habitats that served as potential hibernacula consisted of "caves" (underground passages of sufficient size for human visitation) or a "crevice" (one site; a crack in a bluff face approximately 3 cm wide and no more than 2 m in height).

Bat species	Cave Code	Habitat	County	State	Day-Month	Year	Count
Corynorhinus townsendii	BGC	Cave	Comanche	KS	22-Jan	2012	1
	BSC	Cave	Comanche	KS	21-Jan	2012	2
	CAC	Cave	Comanche	KS	21-Jan	2012	2
	DAC	Cave	Barber	KS	15-Jan	2012	31
	DCC	Cave	Barber	KS	14-Jan	2012	5
	DESC	Cave	Comanche	KS	28-Dec	2011	4
	GEC	Cave	Barber	KS	14-Jan	2012	47
	HC	Cave	Barber	KS	30-Dec	2011	36
	LCC	Cave	Barber	KS	14-Jan	2012	21
	MC	Cave	Woods	OK	14-Jan	2012	70
	PBC	Cave	Comanche	KS	28-Dec	2011	4
	SVC	Cave	Comanche	KS	28-Dec	2011	6
	TAC	Cave	Barber	KS	30-Dec	2011	1
Eptesicus fuscus	DESC	Cave	Comanche	KS	28-Dec	2011	1
	HC	Cave	Barber	KS	30-Dec	2011	1
Myotis velifer	BGC	Cave	Comanche	KS	22-Jan	2012	2
	BSC	Cave	Comanche	KS	21-Jan	2012	2150
	DAC	Cave	Barber	KS	15-Jan	2012	16
	DCC	Cave	Barber	KS	14-Jan	2012	9
	DESC	Cave	Comanche	KS	28-Dec	2011	4791
	GC	Cave	Comanche	KS	21-Jan	2012	358
	GEC	Cave	Barber	KS	14-Jan	2012	1
	HC	Cave	Barber	KS	30-Dec	2011	310
	LCC	Cave	Barber	KS	14-Jan	2012	2
	MC	Cave	Woods	OK	14-Jan	2012	3
	PBC	Cave	Comanche	KS	28-Dec	2011	1213
	SBC	Cave	Comanche	KS	28-Dec	2011	2300
	SVC	Cave	Comanche	KS	28-Dec	2011	5143
	TAC	Cave	Barber	KS	30-Dec	2011	40
	TSC	Cave	Comanche	KS	21-Jan	2012	14

Perimyotis subflavus	BGC	Cave	Comanche	KS	22-Jan	2012	1
	BSC	Cave	Comanche	KS	21-Jan	2012	39
	DAC	Cave	Barber	KS	15-Jan	2012	12
	GEC	Cave	Barber	KS	14-Jan	2012	1
	HC	Cave	Barber	KS	30-Dec	2011	22
	LCC	Cave	Barber	KS	14-Jan	2012	2
	PBC	Cave	Comanche	KS	28-Dec	2011	1
	SBC	Cave	Comanche	KS	28-Dec	2011	1
	TAC	Cave	Barber	KS	30-Dec	2011	1
	TSC	Cave	Comanche	KS	21-Jan	2012	1
(No bats found)	NBBC	Crevice	Barber	KS	30-Dec	2011	0

[a]Data compiled by Dr. William Jensen, Department of Biological Sciences, Emporia State University, Emporia, KS.

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