GENETIC VARIATION WITHIN A SPECIES OF PARASITIC NEMATODE, *SKRJABINGYLUS CHITWOODORUM*, IN SKUNKS

A Thesis

Presented to the Honors Program of

Angelo State University

In Partial Fulfillment of the

Requirements for Highest University Honors

BACHELOR OF SCIENCE

by

ALLIE NEILA DENHAM

May 2020

Major: Biology

GENETIC VARIATION WITHIN A SPECIES OF PARASITIC NEMATODE,

SKRJABINGYLUS CHITWOODORUM, IN SKUNKS

by

ALLIE NEILA DENHAM

APPROVED:

Dr. Loren K. Ammerman

Dr. Robert C. Dowler

Dr. Nicholas J. Negovetich

May 1, 2020

APPROVED:

Dr. Shirley Eoff
Director of the Honors Program

ACKNOWLEDGMENTS

I would like to sincerely thank my research advisor, Dr. Loren K. Ammerman for her continued support and guidance during this project. Dr. Ammerman has sacrificed an immense amount of time to help mold me into the researcher I am today. I have learned so many laboratory techniques and lessons in such a short time working with Dr. Ammerman, and this project would never have been possible without her patience and wonderful guidance. I would also like to extend my gratitude to the Angelo State University Office of Sponsored Projects for awarding me the Undergraduate Faculty-Mentored Grant, as well as the Beta Beta Beta Foundation for additional funding. I want to acknowledge Dr. Robert Dowler and Dr. Nicholas Negovetich for serving as members of my thesis committee and their continued support. Dr. Dowler provided me with excellent resources, contacts to acquire more samples, and always encouraged me. Dr. Negovetich helped me understand the world of parasitology and was always welcoming when I had a question regarding life cycles and research articles. I also want to extend immense appreciation to Hannah Jones, Sydney Decker, Malorri Hughes, and many more students, faculty members, and contacts that have helped me with collecting samples for this project. Another quiet influence has been Jody Casares, who assisted with ordering and shipping materials while offering encouragement through complex situations. Furthermore, I would like to sincerely thank Dr. Shirley Eoff, the Director of the Honors Program, for helping me transfer to Angelo State University during a very difficult time in my life. Dr. Eoff has been a supporter, a friend, and a mentor that I am eternally thankful for. It is especially important to thank Dr. Michael Dixon and Dianna Krejsa, who both hired me to work for the Angelo State Natural History Collection,

where I discovered and learned about *Skrjabingylus*. Lastly, I owe an endless appreciation to my family and friends that have supported me through every step along the way and have given me constant encouragement, love, and hours of listening to phone calls regarding my work. Thank you so much. You all mean the world to me.

ABSTRACT

Carnivores in the families Mustelidae and Mephitidae are prime targets for the nematode genus *Skrjabingylus*. A high prevalence of *Skrjabingylus chitwoodorum* has been observed in the striped skunk, *Mephitis mephitis*. Genetic barcoding studies of other parasitic nematodes have successfully used the cytochrome oxidase I (COI) mitochondrial gene to analyze genetic divergence. We tested the hypothesis that low population structuring occurs within the parasite species due to the high level of gene flow documented in *M. mephitis*. We extracted DNA from 39 samples of *Skrjabingylus* removed from the sinuses of *M. mephitis* and one from *Spilogale putorius interrupta* for amplification and sequencing of COI. Analysis of 492 base pairs confirmed the species as *S. chitwoodorum* and showed low genetic variation (1.0%) within Texas. Supporting our hypothesis, no obvious divergent lineages based on geographic location were recovered within the samples based on Maximum Likelihood analysis and median joining haplotype network analysis.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

The endoparasitic nematode genus, *Skrjabingylus,* is known to target carnivorous hosts in the family Mustelidae, such as weasels, minks, badgers, and otters. Along with mustelids, *Skrjabingylus* also occurs often in the family Mephitidae, which is composed of various skunk species. The parasitic nematode resides primarily within the frontal nasal sinuses of the definitive host (Hansson 1967). The taxonomy of *Skrjabingylus* has an interesting history full of twists and turns. Leuckart first described the parasite in 1842 and named it *Spiroptera nasicola* Leuckart 1842. A parasite found in the lungs of mustelids, *Filaria mustelarum pulmonalis* Rudolphi 1819, now known as *Filaroides bronchialis,* was discovered to often be confused and used interchangeably with *Spiroptera nasicola (*Petrow 1928; Hill 1939) even while both were distinctly different. This confusion caused Petrow to reevaluate the morphological characteristics of both as well as reclassify the genus of *Spiroptera* to *Skrjabingylus* (Petrow 1928), named after the renowned Russian parasitologist, Skrjabin (King 1989). The genus *Spiroptera* still exists but has gone through a separate taxonomic change to now be the genus *Gongylonema.*

Since the reclassification in 1928 five additional species have been discovered. Each species is host-specific, with *Skrjabingylus nasicola* Leuckart 1842 infecting definitive hosts within the genus *Mustela*, comprised of weasels, polecats, and stoats, and the genus *Neovison,* comprised of the minks (Hawkins et al. 2010). *Skrjabingylus chitwoodorum* Hill 1939 is found in skunk genera *Mephitis* and *Spilogale, Skrjabingylus petrowi* Bagenanow and Petrow 1941 and *Skrjabingylus ryjikovi* Kontrimavichus 1961 in the

genus *Martes* (Heddergott et al. 2015), *Skrjabingylus lutrae* Lankester 1972 in river otters (Lankester and Crichton 1972), and the newest addition of *Skrjabingylus santaceciliae* found in *Mephitis macroura* (Carreno et al. 2005). *Skrjabingylus chitwoodorum*, the basis of this thesis, also has had some taxonomic uncertainty. In 1965, Webster named a parasite *Skrjabingylus magnus* when it was found in a Canadian striped skunk, *M. mephitis* (Webster 1965). This name later was amended by Lankester in 1983 when morphological differences could not be found, thus *S. chitwoodorum* remained the true species in the striped skunk and *S. magnus* became a synonym (Lankester 1983).

Morphology has been the main basis of research studies within the genus *Skrjabingylus* and little genetic work has been done thus far. Of the six known species, only four occur in the Americas: *S. nasicola, S. lutrae, S. santaceciliae,* and *S. chitwoodorum* (Lankester 1983; Carreno et al. 2005). The four parasite species have been distinguished through body size, length of the male spicules, a distinctive shape of the distal tip of the spicule, and the host of the parasite (Lankester 1983). The length of male spicules is a common way to differentiate *Skrjabingylus* in the Americas because they are so distinct from one another; *S. nasicola* ranges from 180-232 µm (Lankester 1983), *S. lutrae* ranges from 239-275 µm (Lankester and Crichton 1972), *S. santaceciliae* ranges from 385-428 µm (Carreno et al. 2005), and *S. chitwoodorum* has known ranges of 540-710 µm but up to 890 µm in some studies (Hill 1939; Webster 1965). The coloration of *Skrjabingylus* typically appears bright red. Most hypotheses and photospectrometric experiments suggest that the red coloration occurs due to blood absorption because sample wavelengths are similar to diluted hemoglobin samples (Dougherty and Hall 1955; Theron 1975).

The lifecycle of *Skrjabingylus* species are all very similar and the larval stages have similar sizes and structures. This has caused errors in identifying the parasite. *Skrjabingylus* begins as first-stage larvae in gastropods, mollusks, or fish that can act as intermediate hosts. The intermediate host holds the J_1 larvae that develop to J_3 until the host is either eaten directly by the definitive host or a paratenic host. A paratenic host acts as a bridge to the definitive host and little to no molts will occur in this host. Once in the definitive host, the third-stage larvae enter the gut, burrow through the intestinal wall, and then migrate up localized nerves until they reach the sinuses for maturation (Lankester and Anderson 1971). The cycle repeats when the skunk passes larvae through the gastrointestinal system and then gastropods or other intermediate hosts ingest them in the fecal matter. Another way the cycle repeats is that once inside the nasal sinus, all species (except *S. lutrae*), have been found to be viviparous, and thus release larvae that may exit the sinus through nasal secretions that then enter the pharynx and subsequently the gastrointestinal system, or occasionally will be coughed up (Hansson 1967; Santi and Parker 2012) (Fig. 1).

Originally, very little was known about the spread of *Skrjabingylus* and how it travels through the body of the host, as well as how this parasite is transmitted. The genus is often host-specific and appears to have no direct impact or transmission to humans. Even if transmission occurred from a gastropod or mollusk into humans, the larvae most likely act the same as if they are in a paratenic host with no further progression of growth into the typical life cycle. Experimental studies by Hobmaier (1941), Hansson (1967) and Lankester (1970) provided breakthroughs towards the understanding of the epidemiology, pathology, and transmission of *Skrjabingylus.* Hobmaier (1941) began research on *S. chitwoodorum* to

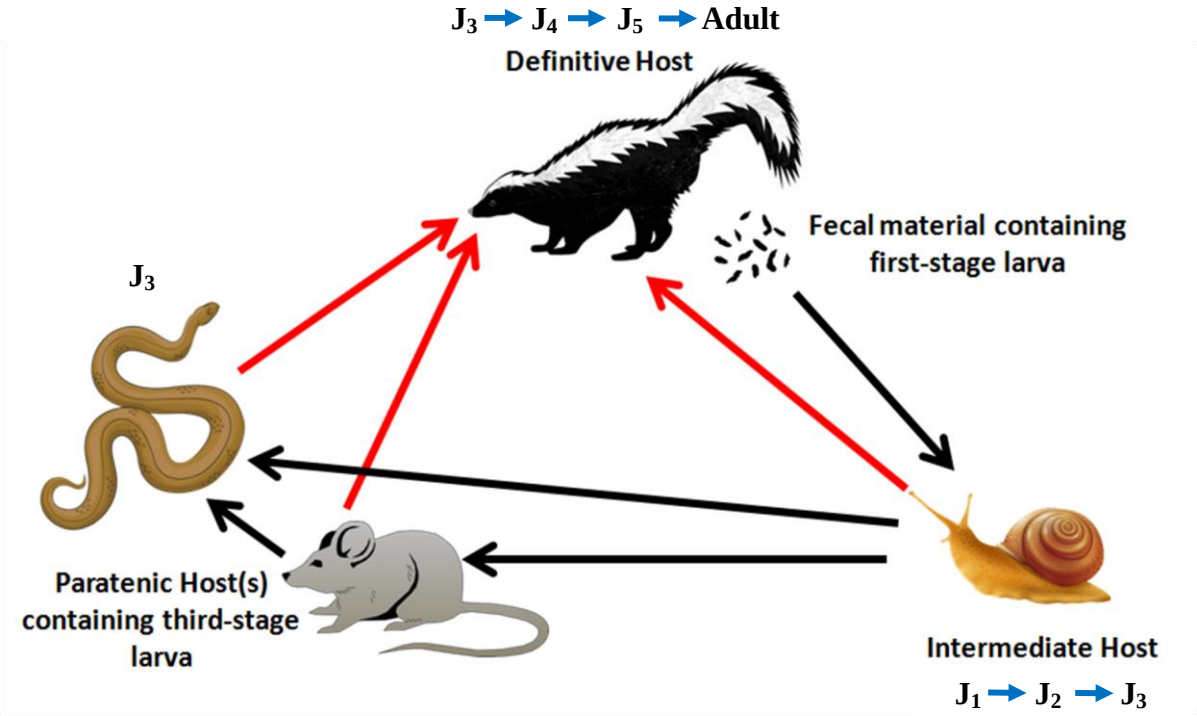

Fig 1. Life cycle of *Skrjabingylus chitwoodorum* in *Mephitis mephitis.* Red arrows correspond to the parasite moving directly to the definitive host and black arrows correspond to the parasite moving to an intermediate or paratenic host. Blue arrows correspond to a molt to the next juvenile (J) phase.

determine if use of an intermediate and/or a paratenic host occurs. His team found larvae in the Pacific garter snake (*Thamnophis sirtalis*), frogs, and other poikilothermic animals such as toads. Along with this, Hobmaier concluded that rats and mice may often serve as accidental carriers, thus becoming prey to animals, such as the garter snake, to therefore carry the larvae to the eventual definitive host. Hansson (1967) experimentally introduced infected skunks with non-infected, as well as introduced non-infected skunks to the feces that came from an infected skunk. She concluded that no transmission of *Skrjabingylus* occurred, therefore showing the importance of an intermediate and/or paratenic host to carrying first-stage larvae and passing them later to the definitive host to become third-stage and begin the journey to the nasal sinuses. Lastly, Lankester (1970) conducted a large-scale project that examined all tissues of infected mink and skunks at various points of initial and late infection, thus showing that the migration and infection of *Skrjabingylus* is far more complex than previously hypothesized. Lankester's studies showed how quickly *Skrjabingylus* begins the primary infection. He concluded that after only 4-5 days post-ingestion the now fifth-stage worms begin to burrow out of the gut walls and at 6 days begin to migrate up the cranial nerves. This breakthrough helped finally pinpoint the exact timeframe and movement of the larval stages that ultimately lead to the transition to worms that grow and thrive in the sinus. Once in the primary host and migration to the sinus occurs, *Skrjabingylus* can then create cranial lesions in its hosts due to cranial pressure, leading to neurological damage (Hansson 1967). Certain studies have also found evidence of *Skrjabingylus* creating meningitis during the migration to the olfactory nerves, which also can create severe blockages and therefore not permit infection in the sinus (Lankester and Anderson 1971).

On an epidemiological level, studies have been done within the genus to show prevalence and intensity. The epidemiological studies stem in two directions: looking at *Skrjabingylus*-damaged skulls from museums (Higdon and Gompper, 2020) versus actually acquiring the parasite directly from sinuses (Heddergott et al. 2015; Hughes et al. 2018). Both epidemiological and morphological studies are important because they help show how widespread *Skrjabingylus* is within Eurasia and the Americas. *Skrjabingylus* are found in certain hosts more often than others. In a study by Heddergott et al. (2015), only 5 of 385 examined *Martes martes* (pine marten) skulls contained nematodes, whereas in a study by Hughes et al. (2018), 290 out of 595 *Mephitis mephitis* (striped skunk) skulls examined had *S. chitwoodorum* present. However, Hughes et al. (2018) postulate that the elevated prevalence of infection was because most of the animals screened were rabies-negative skunks received from the Texas Department of State Health Services. Some of these animals had been submitted because of unusual behavior and suspected rabies infections, but ultimately they were negative for the rabies virus meaning that *Skrjabingylus* might have been responsible for their odd behavior. Skunks in Lankester's study (1970) displayed obvious neurological disturbances after a mere 13 days post-infection. Lankester reported certain skunks experiencing loss of motor coordination and lethargy. These findings are also similar from numerous observations of "dancing" mustelids or mephitids experiencing some sort of episode or epileptic crisis (Lewis 1967; Debrot and Mermod 1980; King 1989).

Studies on prevalence and intensity help answer questions regarding geographical regions or ecosystem preference, as seen in studies from Hughes et al. (2018) in host species *M. mephitis* and Higdon and Gompper (2020) in host species *Spilogale putorius* and

Spilogale gracilis. The study by Hughes et al. (2018) concluded that *S. chitwoodorum* had a bias for the left sinus and also that ecoregion and precipitation was not a determining factor of prevalence. Hughes examined 595 striped skunks and extracted nematodes directly from the sinuses of 48.7% of the skunks. However, the study done by Higdon et al. (2020) concluded after looking at 578 skulls that the prevalence and intensity of *S. chitwoodorum* did have precipitation as a determining factor of prevalence, and they concluded that higher levels of precipitation most likely caused an increase in mollusks and other gastropods that act as intermediate hosts. These differing conclusions could be a result of the species of skunk they examined, the screening method, or their sampling strategies. Higdon et al. (2020) had a larger sampling area that encompassed almost all of the United States due to her team acquiring skulls from various collections whereas Hughes et al. (2018) focused their study on Texas. It is clear that *S. chitwoodorum* is widespread and infects skunks from many different habitat types, genera, and species. There could be underlying genetic differences that allow them to be successful across a variety of habitats but this has not been examined.

Little genetic work has been done within the genus *Skrjabingylus.* A study done by Carreno et al. (2003) sequenced ribosomal DNA genes for a phylogenetic analysis of the superfamily Metastrongyloidea and suggested that the genus *Skrjabingylus* was a sister group to the superfamily. Heddergott et al. (2015) used a barcoding technique using the cytochrome oxidase I gene from 5 individuals to genetically differentiate *S. nasicola* from *S. petrowi.* However, intraspecific variation for *S. chitwoodorum* or any other species within the genus has never been examined. Many studies have reported co-evolution of hosts and nematode parasites. Previous research reported high gene flow in the host of *S. chitwoodorum*, *M.*

mephitis, in an urban population in Texas (Brashear et al. 2015), as well as high gene flow in *M. mephitis* measured across 22,000 square kilometers in Canada, regardless of geographic barriers (Talbot et al. 2012). Due to the studies from Brashear et al. (2015) and Talbot et al. (2012), we expected *Skrjabingylus* to have a similar pattern to *M. mephitis* and exhibit little genetic structuring across the sample area. Generally, the more host-specific the parasite is, a greater similarity in host and parasite phylogenies can be exhibited as we hypothesize to see in *S. chitwoodorum* (Dougherty 1949). In contrast, patterns of mitochondrial sequence evolution in *S. chitwoodorum* could reveal undescribed variation and/or barriers to gene flow. Due to little genetic work, especially an intraspecific study, on *S. chitwoodorum*, this study tested the hypothesis that there is a single lineage of *Skrjabingylus* in *M. mephitis* in Texas. The alternative hypothesis was that the gene sequences would vary and cluster into some pattern of undescribed lineages of *Skrjabingylus*. The objective of this study was to use COI sequences to determine the level of genetic variation within *S. chitwoodorum*, to describe the patterns of variation with respect to geographical sampling locality, and to determine the phylogenetic relationship of *S. chitwoodorum* to other *Skrjabingylus* species.

MATERIALS AND METHODS

Sampling design.— The first step in this project was to collect samples of *Skrjabingylus* from the host skunk, *M. mephitis*. Because a large number of nematodes had been collected by Hughes et al. (2018) and were stored in the Angelo State Natural History Collections (ASNHC), many tissue samples were available for this study. Many of these originated from the rabies lab at Texas Department of State Health Services (DSHS) in Austin. The skulls of rabies-negative skunks were deposited in the ASNHC and were screened for *Skrjabingylus* when the skulls were prepared as voucher specimens. From our freezers and collecting samples, I obtained samples of *Skrjabingylus* from 33 *M. mephitis* from 25 Texas counties (Fig. 2). This allowed a broad coverage in Texas and 5 additional *Mephitis* specimens were acquired from New York (3) and South Dakota (2). We obtained one additional *Skrjabingylus* sample from *Spilogale putorius interrupta* (Eastern Spotted Skunk) from South Dakota that was provided by a fur trapper, and New York samples were provided by Robert J. Rudd. These additional 6 samples were as follows: 3 from Brule County, South Dakota, including the *Spilogale* sample; 1 from Clinton County, New York; and 2 from Franklin County, New York (Table 1).

Selection of genetic marker.— COI rather than nuclear DNA (such as ribosomal genes) was selected to study the population genetics of the nematode because this gene contains more variability for making comparisons as Prosser et al. (2013) found. They created primers to target COI in nematodes and allow for a better intraspecific study than other loci in the past have been able to provide. They used COI to differentiate nematodes belonging to three orders and eight families and successfully constructed a tree with

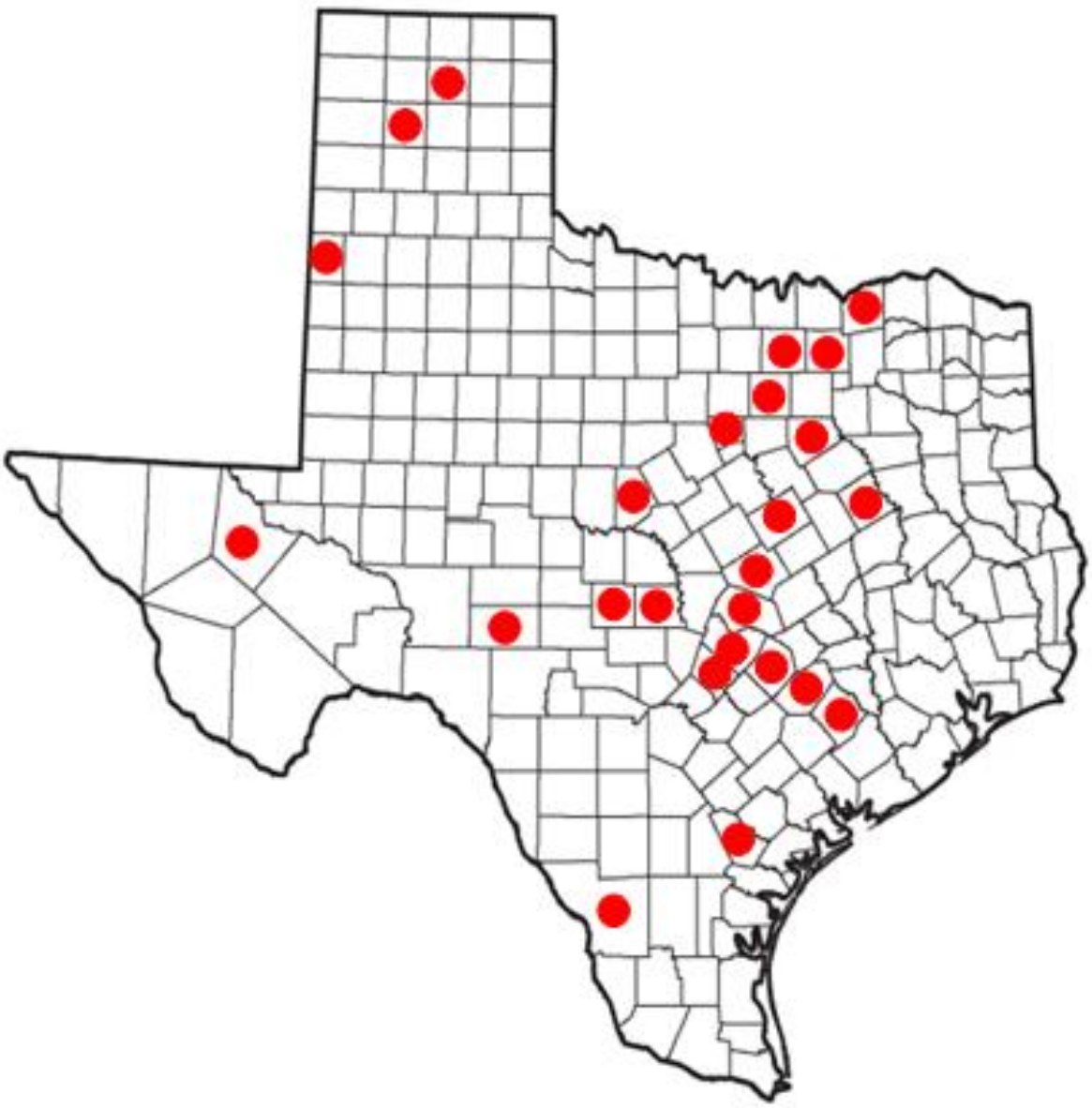

Fig 2. Texas map showing the 25 counties represented in the analysis of *Skrjabingylus* within *Mephitis mephitis* hosts.

Table 1. Information for 39 samples of *Skrjabingylus chitwoodorum* that were used in this study. State and county are the location where the host was collected. Preservation type is given to differentiate which samples had to go through the rehydration process before DNA extraction. Abbreviation ASK denotes material from Angelo State Natural History Collections

Tissue no.	Host Genus	State	County	Preservation
ASK12134	*Mephitis*	New York	Clinton	70% Ethanol
ASK12130	*Mephitis*	New York	Franklin	70% Ethanol
ASK12139	*Mephitis*	New York	Franklin	70% Ethanol
ASK11870	*Spilogale*	South Dakota	Brule	70% Ethanol
ASK14307	*Mephitis*	South Dakota	Brule	Frozen
ASK14311	*Mephitis*	South Dakota	Brule	Frozen
ASK9931	*Mephitis*	Texas	Bailey	Frozen
ASK9844	*Mephitis*	Texas	Bastrop	Frozen
ASK9752	*Mephitis*	Texas	Bee	Frozen
ASK13294	*Mephitis*	Texas	Bell	70% Ethanol
ASK13265	*Mephitis*	Texas	Brown	70% Ethanol
ASK12039	*Mephitis*	Texas	Collin	Frozen
ASK9908	*Mephitis*	Texas	Collin	Frozen
ASK9790	*Mephitis*	Texas	Colorado	Frozen
ASK9877	*Mephitis*	Texas	Denton	Frozen
ASK9913	*Mephitis*	Texas	Denton	Frozen
ASK13296	*Mephitis*	Texas	Ellis	70% Ethanol
ASK13295	*Mephitis*	Texas	Fannin	70% Ethanol
ASK9749	*Mephitis*	Texas	Fayette	Frozen

Tissue no.	Host Genus	State	County	Preservation
ASK13596	*Mephitis*	Texas	Freestone	70% Ethanol
ASK13245	*Mephitis*	Texas	Hays	70% Ethanol
ASK13244	*Mephitis*	Texas	Hood	70% Ethanol
ASK13619	*Mephitis*	Texas	Hutchinson	70% Ethanol
ASK7426	*Mephitis*	Texas	Hutchinson	Frozen
ASK13593	*Mephitis*	Texas	Llano	70% Ethanol
ASK13618	*Mephitis*	Texas	Llano	70% Ethanol
ASK9770	*Mephitis*	Texas	Llano	Frozen
ASK9779	*Mephitis*	Texas	Llano	Frozen
ASK9975	*Mephitis*	Texas	Mason	Frozen
ASK9868	*Mephitis*	Texas	McLennan	Frozen
ASK13275	*Mephitis*	Texas	Potter	70% Ethanol
ASK9774	*Mephitis*	Texas	Potter	Frozen
ASK13208	*Mephitis*	Texas	Reeves	70% Ethanol
ASK9865	*Mephitis*	Texas	Sutton	Frozen
ASK13259	*Mephitis*	Texas	Tarrant	70% Ethanol
ASK9750	*Mephitis*	Texas	Travis	Frozen
ASK13278	*Mephitis*	Texas	Webb	70% Ethanol
ASK9784	*Mephitis*	Texas	Williamson	70% Ethanol
ASK9746	*Mephitis*	Texas	Williamson	Frozen

96 samples to show the reliability of barcoding COI across a broad range of nematode families. In a similar study by Derycke et al. (2010), COI was used in 41 free-living marine nematode species to discriminate one morphological species from another. They only were unable to differentiate two out of 41 species (Derycke et al. 2010). This meant that COI showed variability in DNA sequencing and would likely differentiate *S. chitwoodorum* lineages from one another. Furthermore, generating COI sequences for this study allowed comparisons to other *Skrjabingylus* species, such as those studied by Heddergott et al. (2015) in martens. Their study found 74 polymorphic sites in COI of 3 sequences of *S. petrowi* from two *Martes martes* (pine marten) and one *M. foina* (stone marten) compared to *S. nasicola* from 2 *M. putorius* (European polecat) collected in various states within Germany. We used primers NemF2_t1: ARAGATCTAATCATAAAGATATYGG and NemR2_t1: AWACYTCWGGRTGMCCAAAAAAYCA (Prosser et al. 2013) to amplify a 661-base pair (bp) fragment of COI.

DNA Extraction.— DNA was extracted from individual *S. chitwoodorum* with the Qiagen DNeasy Blood and Tissue Kit and the following methodology: the sample of tissue was added to 180 µL ATL buffer and 20 µL proteinase K. The sample was vortexed and incubated for 48 hours (56°C) to lyse completely. After 24 hours of incubation, I added 20 µL proteinase K. After 48 hours, 200 µL buffer AL was added and the tube was vortexed. The samples were then placed into 70°C for 5.5 hours. After 5.5 hours, 200 µL of ethanol was added and vortexed, followed by the contents being transferred to a spin column and the steps in the manufacturer's protocol were followed. Lastly, the elution occurred with 50 µL AE elution buffer. If the sample was preserved in ethanol, the worm was soaked three times

in 1X PBS (phosphate buffered saline) for 10 minutes each before DNA extraction occurred in order to rehydrate the sample. After extraction occurred, purity and concentration values were measured using the NanoDrop Lite Spectrophotometer.

PCR amplification and sequencing.— The final reaction conditions used to amplify COI were as follows: 0.25-2 µL template DNA ranging from 1.2-110 ng, 0.08 U *Taq* polymerase (New England Biolabs, Ipswich, MA), 0.8mM of each dNTP (Thermo Fisher Sci., Waltham, MA), 1X standard reaction *Taq* buffer (New England Biolabs, Ipswich, MA), 2 mM $MgCl_2$ (New England BioLabs, Ipswich, MA), 0.16 µM of each forward and reverse primers (Alpha DNA, Montreal, Quebec, Canada), and RNase free water as needed to reach a final volume of 12.5µL. The COI reactions were amplified using the following thermalcycler profile modified from Heddergott et al. (2015): a denaturing step of 94°C for 1 minute, followed by 40 cycles of 94°C for 40 seconds, annealing at 51°C for 40 seconds, 72°C for 1 min, and a final extension of 72°C for 5 min. Only the first elutions were used for PCR due to a majority resulting in a higher concentration of DNA compared to the second elution.

Products were analyzed and verified using gel electrophoresis and a FastRuler middle range ladder (Thermo Fisher Sci., Waltham, MA) to determine gene amplification. Quantification of PCR product was obtained using a fluorometric Qubit HS Assay kit (Thermo Fisher Sci., Waltham, MA). Samples with a minimum of 80 ng were then purified with ExoSAP-IT Express PCR Product Cleaning Reagent (Thermo Fisher Sci., Waltham, MA) following the manufacturer's protocol. The purified samples were then shipped to the Texas A&M Corpus Christi Genomic Core Sequencing Lab for Sanger sequencing of both DNA strands.

Sequence analysis.— Once sequences were returned, I carefully analyzed them using Sequencher® version 5.4.6 DNA sequence analysis software (Gene Codes Corporation, Ann Arbor, MI USA) to assemble contigs from forward and reverse sequences and create consensus sequences for each individual. After confirming that each sequence translated correctly, I exported the sequences and then aligned them in MEGA X (Kumar et al. 2018) using the MUSCLE algorithm. A phylogenetic analysis was conducted with MEGA X (Kumar et al. 2018) using the gene sequences of *S. chitwoodorum* that were generated and two other species of *Skrjabingylus* from GenBank, *S. petrowi* (KP724692-KP724694) and *S. nasicola* (KP724695-KP724696). A phylogenetic tree was created on MEGA X using Maximum Likelihood criteria and the best-fitting DNA model, Hasegawa-Kishino-Yano + G (HKY+G). Bootstrap analysis (1000 replicates) was conducted to evaluate the support of each branch. This was for the first level of analysis in which I performed a phylogenetic analysis among species of *Skrjabingylus*.

I calculated genetic divergence based on COI both within *S. chitwoodorum* (among Texas counties) and between Texas and samples from New York and South Dakota. Divergence among the three *Skrjabingylus* species was also calculated using Kimura-2 parameter (K2P) for pairwise-distance computation in MEGA X (Kumar et al. 2018). K2P was used for genetic divergence analysis because it was the model that had the closest substitution rate and pattern to HKY+G. A median joining haplotype network analysis was performed using PopArt to identify haplotypes and further show possible intraspecific patterns (Leigh and Bryant 2015). An actual measurement of gene flow was not calculated in this analysis because we did not obtain a large sample size to form distinct populations that

mirror geography. We instead combined all samples into one cluster to evaluate results with respect to geographic origin.

RESULTS

Primer optimization. — Originally the thermocycler protocol from Heddergott et al. (2015) was used to amplify the COI gene through our barcoding technique. A double banding pattern occurred after the first PCR and electrophoresis gel. The annealing and extension temperatures were kept the same but 40 cycles were used instead of 35. This modification resulted in successful amplification. No significant pattern was seen between successful amplification and correlation to purity ratio and/or concentration of template DNA. Frozen samples appeared to amplify at a slightly higher rate with 21 out of 39 samples (53.8%) producing PCR product. Samples preserved in alcohol typically had lower purity values than frozen samples but still amplified compared to lower levels of frozen samples that would not produce reliable or bright enough bands on the gels.

Cytochrome oxidase I interspecific analysis. — COI sequences were obtained from 39 samples of *Skrjabingylus* from 38 *M. mephitis* hosts and 1 *S. p. interrupta* host. Thirty-three of these samples encompassed 25 counties in Texas, while the remaining 6 samples were from out-of-state. The Maximum Likelihood analysis of the fragment of COI composed of 492 base pairs recovered 3 distinct lineages: *S. petrowi*, *S. nasicola*, and a lineage composed of all *S. chitwoodorum* (Fig. 3). Bootstrap analysis using 1000 replicates significantly supported that the samples comprised a distinct lineage divergent from the other two species of *Skrjabingylus*. Within *S. chitwoodorum* samples, there was no obvious pattern of close geographic relationships other than a branch of 4 out-of-state samples, 3 from New York and 1 from South Dakota that were placed at the base of the tree and had bootstrap support of 76% (Fig. 3). The sample of *S. chitwoodorum* from *Spilogale* (ASK11870)

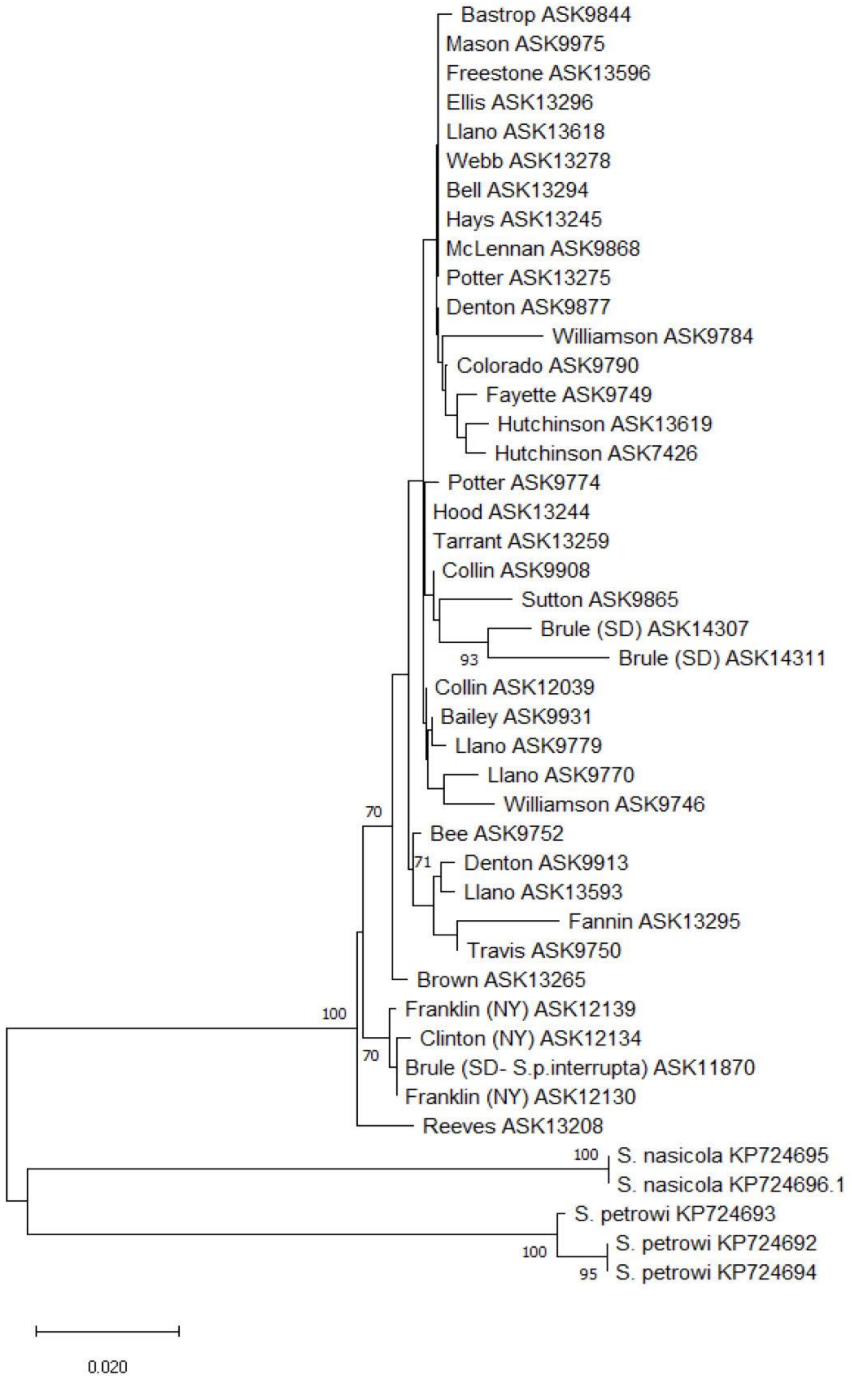

Fig 3. Maximum likelihood phylogenetic tree of 498 base pair fragment of the cytochrome oxidase I gene for 44 samples of *Skrjabingylus.* Maximum Likelihood analysis was performed using the best-fitting model, Hasegawa-Kishino-Yano of DNA substitution with Gamma distribution. Bootstrap values are based on 1000 replicates and shown on branches with greater than a value of 70.

collected in South Dakota clustered closely with other samples that were collected from *M. mephitis*. However, the other two samples from South Dakota were sister taxa (supported by a 92% bootstrap value) and clustered with a worm from Sutton County, Texas. For the most part, among the samples from Texas there were no well supported lineages that linked samples from the same county of collection. Among all three species, 125 polymorphic sites were documented. *S. chitwoodorum* was 14.2% divergent from the outgroup taxon *S. petrowi* and 14.4% divergent from *S. nasicola.* A 15.9% difference was observed between *S. nasicola* and *S. petrowi* (Table 2).

Cytochrome oxidase I intraspecific analysis.— In total, 58 polymorphic sites occurred within the 39 *S. chitwoodorum* samples included in this study, 20 of which were parsimony-informative and 38 were singleton sites. The overall average variation within the 33 Texas samples was 1.00% genetic divergence (Table 2). The genetic diversity slightly increased when the 6 out-of-state samples were added into the analysis, showing a 1.29% average divergence. The highest divergence within Texas samples was 3.78% between Fannin (ASK13296) and Williamson (ASK9784) counties. When comparing Texas samples to the 6 out-of state samples, the highest variation was 5.24% between Fannin (ASK13296) County in Texas and Brule (ASK14311) County in South Dakota.

Haplotype network analysis. — In total, there were 22 COI haplotypes in *S. chitwoodorum* with 3 haplotypes being shared by 2 or more samples (Fig. 4). The Bell County (ASK13294) haplotype was the most common and was shared with 10 others (Table 3). Bailey County (ASK9931) shared a haplotype with 5 other samples from Texas.

Table 2. Average Kimura 2-parameter distance among outgroup taxa *Skrjabingylus nasicola* and *Skrjabingylus petrowi* and populations of *Skrjabingylus chitwoodorum* from *Mephitis mephitis* in various states based on 492 bases of COI.* Sample sizes are listed in parentheses.

	S. nasicola	***S. petrowi***	**Texas**	**South Dakota**	**New York**
***S. nasicola* (2)**	**0.008**				
***S. petrowi* (3)**	0.159	**0.000**			
Texas (33)	0.144	0.147	**0.010**		
South Dakota (3)	0.159	0.157	0.023	**0.030**	
New York (3)	0.140	0.134	0.018	0.023	**.002**

* One of the samples from South Dakota was from *Spilogale*

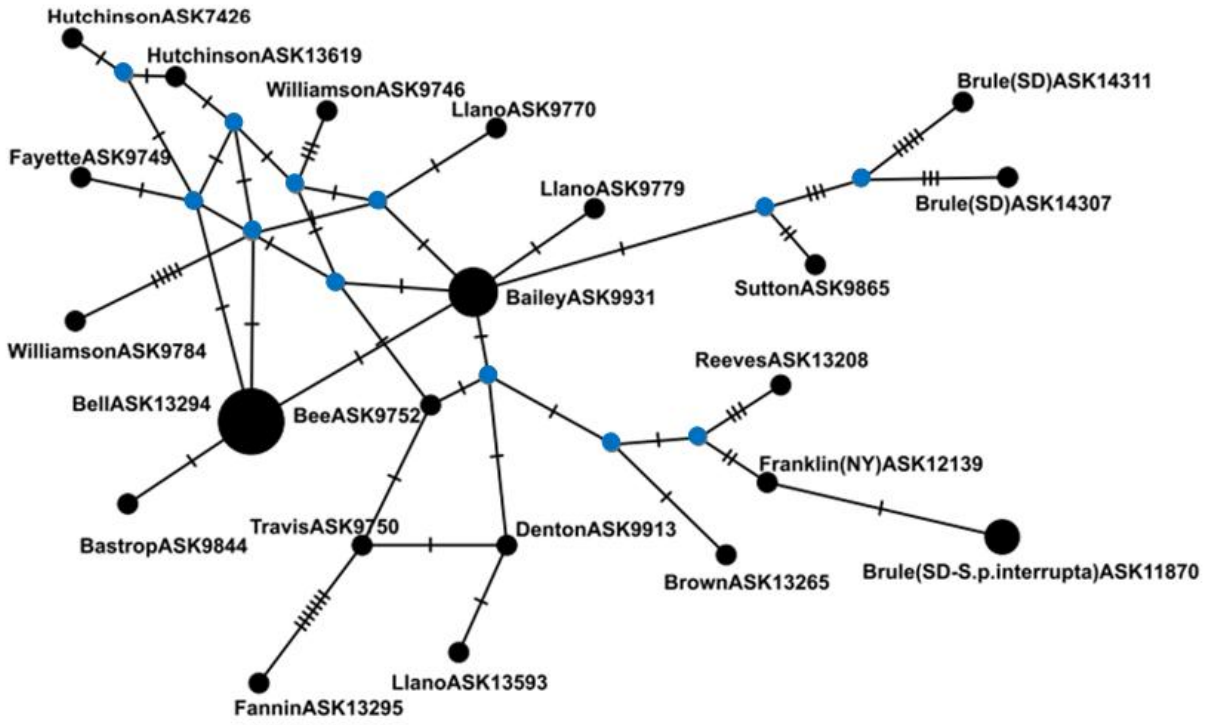

Fig 4. Median joining network showing the relationships among haplotypes of 44 samples of *Skrjabingylus chitwoodorum* from hosts *Mephitis mephitis* and *Spilogale putorius interrupta* using COI mtDNA. Small blue circles represent hypothetical haplotypes and ticks on branches represent number of mutational steps.

Table 3. The three most common COI haplotypes that had multiple samples with the same mutational changes. The top listed county is the county listed on the median joining network analysis (Fig. 4).

Haplotype 1	Haplotype 2	Haplotype 3
Bell ASK (13294)	Bailey (ASK9931)	Brule (SD) (ASK11870)*
Denton (ASK9877)	Collin (ASK12039)	Clinton (NY) (ASK12134)
Ellis (ASK13296)	Collin (ASK9908)	Franklin (NY) (ASK12130)
Freestone (ASK13596)	Hood (ASK13244	
Hays (ASK13245)	Potter (ASK9774)	
Llano (ASK13618)	Tarrant (ASK13259)	
Mason (ASK9975)		
McLennan (ASK 9868)		
Potter (ASK13275)		
Webb (ASK13278)		

* Sample from *Spilogale.* All others were from *Mephitis mephitis* hosts

Interestingly, the one sample of *Skrjabingylus* from the host species *Spilogale* in Brule County (ASK11870) of South Dakota shared a haplotype with two other counties, Clinton and Franklin County in New York (ASK12134 and ASK12130). Nucleotide diversity, represented by π, was 3.05806E+06. Mutational steps ranged between 1 to 7 changes with the highest occurring between Travis County (ASK9750) and Fannin County (ASK13295).

DISCUSSION

This project strived to bring light to the highly prevalent, yet severely understudied parasitic nematode, *S. chitwoodorum*. The world of parasitology and mammalogy have been forced to rely on morphological characteristics to distinguish genera and species for centuries, but now with molecular advancements and continuously evolving research the possibilities of new discoveries and additions to previous studies are rapidly increasing. This study used genetic data to show that *S. chitwoodorum* is a distinct lineage present in the three U.S. states from which samples were acquired. In addition, there was a low level of variation in COI, even if samples were separated by long distances. Lastly, the analysis completed in this study showed a lack of a distinct pattern of genetic lineages that mirror geography, which suggests high levels of gene flow. Thus, these data provided support for the original hypothesis.

Only two known species of *Skrjabingylus* are present on GenBank. The sequences gathered in this project will be added to represent *S. chitwoodorum* and to officially have three of the six species included in the nucleotide database. Having two other species of *Skrjabingylus* available on GenBank allowed us to perform an interspecific study in order to differentiate *S. chitwoodorum.* Each of our 39 samples clustered together in their own clade clearly separate from other *Skrjabingylus* species. A 14.2%-14.6% genetic difference of *S. chitwoodorum* from both outgroup species is similar to that of Heddergott et al. (2005) who reported 12.5% sequence divergence between *S. nasicola* and *S. petrowi* for COI. We used a slightly smaller fragment in our analysis; therefore, a slight percentage difference could occur depending on how many of their observed polymorphic sites we ended up including in

our final fragment. The separate clade of *S. chitwoodorum* then allowed us to take a deeper look into intraspecific genetic variability within Texas.

Skrjabingylus chitwoodorum sequences from across the country remained incredibly conserved as seen by an average of 1.29% genetic variation and haplotypes from distant sites were shared. This finding supports our original hypothesis in which *S. chitwoodorum* would experience low genetic structuring due to studies that found high gene flow in their *Mephitis* host (Talbot et al. 2012; Brashear et al. 2013). Other parasitic studies have often found similar results with parasites experiencing the same evolutionary patterns as their hosts. One example is *Baylisascaris schroederi*, a nematode found in the stomach of wild giant pandas in China (Zhou et al. 2013). Similar to our results of a 1.29% average divergence among our three states (TX, SD, and NY) they found low diversity (2.80%) and a significant, high level of gene flow in 44 samples from three mountain ranges using a barcoding method on the cytochrome b mitochondrial gene. Numerous factors have been hypothesized for low genetic diversity such as a low DNA mutation rate, frequent movement of the hosts, or a combination of both that could be driving the patterns that we recovered in this study of *Skrjabingylus* (Zhou et al. 2013). A separate study on the plant-parasitic nematode, *Longidorus poessneckensis,* also showed similar findings (Kornobis et al. 2017). After an analysis on 16 populations, COI revealed low variability (0–2.4%) while nicotinamide dehydrogenase subunit 4 (*nad4)* showed a higher variability (0–7.6%). These two contrasting results once more show that the particular loci being analyzed may play a role in determining intraspecific variability. Our results show a blend of both of these, since our highest point of divergence was 5.24% when comparing a South Dakota sample to Fannin County in Texas.

More loci should therefore be analyzed like the control region of mitochondrial DNA in future studies to have a further understanding of intraspecific variation within *S. chitwoodorum.*

One sample of *Skrjabingylus* (ASK11870) from the host species *Spilogale putorius* in Brule County of South Dakota shared a haplotype with two other counties, Clinton and Franklin County in New York (ASK12134 and ASK12130). It is important to note that the *Spilogale* sample was processed separately during the DNA extraction and PCR process to avoid contamination. This is an interesting finding because it shows that although found in different skunk genera, *S. chitwoodorum* showed little to no variation and expressed identical mutations even with samples separated by approximately 2,000 km. A shared haplotype between two genera of hosts provides a future research endeavor to see how truly host-specific *S. chitwoodorum* is compared to the other species of *Skrjabingylus,* which generally (except *S. nasicola*) reside only within one genus or species (Tumlison and Tumlison 2019).

Various limitations were present in this study. Although many samples were acquired from a rabies lab in Austin, Texas, it would have been beneficial to reach out to more labs across Texas. Attempts were made with the DSHS lab in El Paso, but no samples were sent. Acquiring more samples from the same regions would have allowed us to form populations and therefore be able to perform additional population genetic analyses. With populations we would have been able to assess F_{st} values and further discuss haplotypes within subpopulations, as well as add a statistical approach to our analysis. Only one sample from host genus *Spilogale* was present in our collection, so acquiring additional samples would have strengthened our analysis on the one *Spilogale* sample that formed a haplotype with two

Texas counties. The Covid-19 pandemic also greatly impacted access to the lab and therefore hindered me from resequencing additional samples that did not provide good sequences in our first trial. If I had been able to assess and redo the failed sequences our overall sample size would have been quite larger. Another issue that occurred was that four sequences did not match with *S. chitwoodorum*; therefore, upon blasting the sequence into GenBank we realized that our primers amplified COI from *M. mephitis.* This finding was interesting because it meant that the primers used by Prosser et al. (2013) not only were successful among a range of nematodes, but also possibly vertebrates. There is an obvious sensitivity of the primer to amplifying contaminating host DNA versus the parasite DNA that should be explored in future research efforts.

Future studies on species in the genus *Skrjabingylus* should include different loci to see if the trend of low diversity occurs. Other possible loci that have been explored within *Skrjabingylus* without having to create primers is the small-subunit ribosomal RNA (SSU rRNA) or large-subunit ribosomal RNA (LSU rRNA) used in a study from Carreno et al. (2003). Their study primarily focused on an analysis of the Metastrongyloidea superfamily to better differentiate various strongylid nematode genera. Although somewhat successful at separating genera into sister groups and determining relatedness, Carreno was unable to resolve exactly where the only *Skrjabingylus* representative he included, *S. chitwoodorum*¸ fit within the metastrongyloid families. SSU and LSU are therefore highly conserved genes and using primers for these two regions would most likely not provide a clear distinction at the species level. It would be more beneficial to create or acquire primers for an area of mitochondrial genes that exhibit faster changes than SSU, LSU, or COI, such as the control

region (D-loop). A full nuclear genome study could also be beneficial since no known full genome projects have occurred with *Skrjabingylus*

Mitochondrial barcoding has several limitations such as the risk of heteroplasmy and nuclear pseudogenes of mitochondrial origin (NUMTs) that create the possibility of obtaining samples of different haplotypes within an individual, rather than only finding haplotypes within a population (Rubinoff et al. 2006). Finding nucleotide differences between different mitochondrial haplotypes within single individuals are common and have even been found in the nematode species, *Caenorhabditis elegans* (Tsang and Lemire 2002). NUMTs are typically characterized by the following features: frameshift mutations, indels, or stop codons present in sequences, or PCR amplification that constantly produces more than one band or different bands (Zhang and Hewitt 1997). Pseudogenes, like NUMTs, often result in a sample being placed at the bottom of a constructed phylogenetic tree analysis. Reeves County (ASK13208) is an example of a county that was placed at the base of the tree and had the potential to be a NUMT. In order to limit the chance of a NUMT present in this study, the protein translation of each sequence used in this analysis was examined and there were no frameshift mutations or stop codons in the fragments used; therefore, I feel confident they do not represent NUMTs or pseudogenes.

Future research efforts on *Skrjabingylus* have the possibility to combine numerous fields of biology. One such project could be to perform a genetic study on the newest species, *S. santaceciliae* to confirm that this species is genetically different and not experiencing phenotypic plasticity that caused morphological changes since the parasite occurs in a different *Mephitis* species as well as a dramatically different climate in Costa Rica (Carreno

et al. 2005). Their discovery was based on morphological observations of the size of both sexes as well as spicule length and tip shapes of the males. The spicule is a prominent projection but is much smaller in *S. santaceciliae* compared to *S. chitwoodorum*. In particular, *S. chitwoodorum* has longer body measurements and egg-shaped spicule tips whereas *S. santaceciliae* remains shorter in body length and spicule length, and appears to have no egg-shaped tips. *Skrjabingylus chitwoodorum's* body measurements have wider ranges compared to the other species and they overlap with measurements of *S. santaceciliae*. For example, the length of the gubernaculum in males have two observed ranges of 83-100 µm (Lankester 1983) and 72-88 µm (Hill 1939) in *S. chitwoodorum,* whereas *S. santaceciliae's* range is 75-90 µm. Other examples of overlapping body measurements are the distance of excretory pore from the anterior end of males and the distance of anus from the posterior end in females. Although certain measurements appear to show *S. santaceciliae* as a smaller version of *S. chitwoodorum,* the overlapping seen in particular measurements help show a need for a deeper analysis. A molecular analysis including *S. santaceciliae* and *S. chitwoodorum* could provide additional support for this newly described lineage.

Additional Parasite Implications

Agriculture. — Nematodes parasitize many other forms of life apart from mammals, such as in plants. Over 4,100 species of plant-parasitic nematodes have been discovered that play a costly role in agriculture (Bernard et al. 2017). An estimated $80-$180 billion dollars of revenue is lost each year due to crop damage caused by nematodes globally (Bernard et al. 2017). These parasites often diminish essential water from entering a plant's system and

therefore bring death to the host. A similar reaction has been observed in skunks where migrating worms actually create severe blockages in the spinal column and therefore cause numerous pathological adversities, such as meningitis, to the skunk and even death (Lankester and Anderson 1971).

Vaccination. — Analyses have shown that over a quarter of humans are infected with a parasitic nematode or flatworm (Coghlan et al. 2019). The phylum Nematoda, where *Skrjabingylus* resides, is part of the superphylum Ecdysozoa that has five major clades. Within the five major clades, four contain parasites with the capability to infect humans (Coghlan et al. 2019). No vaccines are currently available against parasites transmitting to humans, and very few are available for livestock (Hewitson and Maizels 2014). Studying the genome of parasites greatly improves our chances of creating certain vaccines if we are able to differentiate and target sections of genes that control metabolism or protein development.

State Health Department and Rabies Testing. — The infection of *S. chitwoodorum* in skunks has the potential for economic effects, especially towards State Health Departments and rabies labs. Many of the skunks collected in this study came directly from rabies labs after the public submitted a skunk acting strangely, which was then tested for rabies. This strange behavior could have been caused from the painful lesions created by the parasite. The pathogenesis of *Skrjabingylus* can therefore have economic impacts on testing methods of the rabies labs. In a study done on Texas skunk collections by rabies labs, over 1,900 skunks were collected in 2002 with approximately 800 testing positive and various years also peaked above 1,000 total skunks collected for testing (Oerteli et al. 2009). If the parasite does in fact cause neurological effects that ultimately hinder movement and mimic odd behavior caused

iny the rabies virus then a spike in infection could greatly impact labs across Texas. The impact comes from the fact that although *M. mephitis* seem to have a high prevalence of *S. chitwoodorum,* the genus *Skrjabingylus* has the capability to be found in mustelids, which mean weasels, minks, otters, and other North American animals can experience the same infection and therefore potentially impact rabies labs workload. If transmission rates suddenly changed then a spike in cases could occur. This highlights an importance in future research endeavors that might benefit the animals and the workload of rabies labs if a PCR test were created to screen the animal for *Skrjabingylus*, rather than sacrifice the animal and unnecessarily screen for rabies. Alternatively, a rabies PCR screening would be even more beneficial because it can have an impact on an even wider range of animals, including domestic pets, bats, and skunks.

Climate change. — Understanding disease transmission is critical, especially when numerous factors have the capability of impacting the rate of transmission or prevalence of a disease. Global warming and climate change could play a role in the spread of *Skrjabingylus* and other parasites due to precipitation levels and temperature changes possibly being a cause for higher or lower prevalence (Cook 1992). Recent research on *S. chitwoodorum* in the eastern and western spotted skunks, *Spilogale putorius* and *Spilogale gracillus*, showed through mathematical modeling that precipitation in the year prior of infection and prior to host-collection impacted presence and intensity (Higdon and Gompper 2020). Precipitation could lead to a higher population of gastropods in the environment infected with J_1 larvae and transmit J_3 larvae to more definitive hosts or be carried in paratenic hosts. As temperatures rapidly change due to climate change, the ideal environment for *Skrjabingylus* may be

impacted. Species such as *S. nasicola* are typically found more often in colder and wetter climates within Europe, whereas *S. chitwoodorum* seems to thrive well in a wide range of environments as seen in prevalence studies over the years (Hughes et al. 2018, Higdon et al. 2020). Little research has been able to determine what body temperatures are ideal for each species, thus leaving precipitation levels and overall climate to be the main factors to explain the spread and survivability of *Skrjabingylus.*

Conclusion. — Overall, many more studies are necessary to fully understand biodiversity within the genus *Skrjabingylus.* The addition of more genetic studies not only will help answer additional intraspecific and interspecific questions but also give rise to the capability of better distinguishing species within *Skrjabingylus* and their possible intermediate and paratenic hosts that aide us in better understanding the complicated and intricate life cycle of this parasitic nematode.

LITERATURE CITED

BERNARD, G. C., M. EGNIN, AND C. BONSI. 2017. The impact of plant-parasitic nematodes on agriculture and methods of control. Pp. 121–151 in Nematology: concepts, control, and diagnosis (M. M. Shah and M. Mahamood, eds.). IntechOpen. London, United Kingdom. doi: 10.5772/intechopen.68958

BRASHEAR, W., L. K. AMMERMAN AND R. C. DOWLER. 2015. Short-distance dispersal and lack of genetic structure in an urban striped skunk population. Journal of Mammalogy 96:72–80. doi:10.1093/jmammal/gyu004.

CARRENO, R. A., AND S.A. NADLER. 2003. Phylogenetic analysis of the Metastrongyloidea (Nematode: Strongylida) inferred from ribosomal RNA gene sequences. Journal of Parasitology 89:965–973.

CARRENO R. A., K. E. REIF, AND S. A. NADLER. 2005. A new species of *Skrjabingylus* Petrow, 1927 (Nematoda: Metastrongyloidea) from the frontal sinuses of the Hooded Skunk, *Mephitis macroura* (Mustelidae). Journal of Parasitology 91:102–107.

COGHLAN, A., R. TYAGI, J. A. COTTON, N. HOLROYD, B. A. ROSA, I. J. TSAI, D. R. LAETSCH, ET AL. 2019. Comparative genomics of the major parasitic worms. Nature Genetics 51:163–174. https://doi.org/10.1038/s41588-018-0262-1

COOK, G. C. 1992. Effect of global warming on the distribution of parasitic and other infectious diseases: a review. Journal of the Royal Society of Medicine 85:688–691.

DEBROT, S., AND C. MERMOD. 1980. Cranial helminth parasites of the stoat and other mustelids in Switzerland. Worldwide Furbearer Conference Proceedings 11:690–705.

DERYCKE, S., J. VANAVERBEKE, A. RIGAUX, T. BACKELJAU, AND T. MOENS. 2010. Exploring the use of cytochrome oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes. PLoS ONE 5: e13716. doi:10.1371/journal.pone.0013716

DOUGHERTY, E. 1949. The phylogeny of the nematode family Metastrongylidae Leiper, [1909]: A correlation of host and symbiote evolution. Journal of Parasitology 39:222–234. doi:10.1017/S0031182000083785

DOUGHERTY, E. C. AND E. R. HALL. 1955. The biological relationships between American weasels (genus *Mustela*) and nematodes of the genus *Skrjabingylus* Petrov, 1927 (Nematoda: Metastrongylidae), the causative organisms of certain lesions in weasel skulls. Revista Iberica de Parsasitologia 15:531–576.

HANSSON, I. 1967. Transmission of the parasitic nematode *Skrjabingylus nasicola* (Leuckart 1842) to species of *Mustela* (Mammalia). Oikos 18:247–252.

HAWKINS C. J., P. STUART, M. HEDDERGOTT, AND C. LAWTON. 2010. Sinus worm (*Skrjabingylus nasicola* (Leuckart, 1842)) infection in American mink (*Mustela vison* Schreber, 1777) in Ireland. The Irish Naturalists' Journal 31:108–112.

HEDDERGOTT, M., F. MÜLLER, AND A. C. FRANTZ. 2015. Prevalence and molecular identification of the sinus worm *Skrjabingylus petrowi* (Nematoda: Metastrongyloidea) from *Martes* spp. in Germany. Parasitology Research 114:2053–2061. doi:10.1007/s00436-015-4388-0

HEWITSON, J. P. AND R. M. MAIZELS. 2014. Vaccination against helminth parasite infections. Expert Review of Vaccines 13:473–487. doi: 10.1586/14760584.2014.893195 P

HILL, W. C. 1939. The nematode *Skrjabingylus chitwoodorum* n. sp. from the skunk. Journal of Parasitology 25:475–478.

HIGDON, S. D., AND M. E. GOMPPER. In Press. Prevalence and severity of skunk cranial worm (*Skrjabingylus chitwoodorum)* damage increase with precipitation in spotted skunks (*Spilogale* spp.). Journal of Wildlife Diseases. doi.org/10.7589/2019-07-182

HOBMAIER, M. 1941. Extramammalian phase of *Skrjabingylus chitwoodorum* (Nematoda). Journal of Parasitology 27:237–239.

HUGHES, M. R., N. J. NEGOVETICH, B.C. MAYES, AND R. C. DOWLER. 2018. Prevalence and intensity of the sinus roundworm (*Skrjabingylus chitwoodorum)* in rabies-negative skunks of Texas, USA. Journal of Wildlife Diseases 54:85–94. doi: 10.7589/2017-02-023

KING, C. M. 1989. The natural history of weasels and stoats. Comstock Publishing Associates, Cornell University Press: New York.

KONTRIMAVICHUS, V. L. 1961. The helminth fauna of *Martes flavigula* and a description of *Skrjabingylus ryjikovi* n. sp. (Pseudaliidae: Nematoda). Helminthologia 3:168–173.

KORNOBIS, F. W., S. A. SUBBOTIN, AND S. KUMARI. 2017. Molecular characterization of plant parasitic nematode *Longidorus poessneckensis* Altherr, 1974 (Nematoda: Longidoridae). European Journal of Plant Pathology 151:791–802. doi: 10.1007/s10658-017-1417-0

KUMAR, S., G. STECHER, M. LI, C. KNYAZ, AND K. TAMURA. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549.

LANKESTER, M. W. 1970. The biology of *Skrjabingylus* spp. (Metastrongyloidea: Pseudaliidae) in mustelids (Mustelidae). Ph.D dissertation, University of Guelph. Guelph, Ontario.

LANKESTER, M. W. 1983. *Skrjabingylus* Petrov, 1927 (Nematoda: Metastrongyloidea) emended with redescriptions of *S. nasicola* (Leuckart, 1842) and *S. chitwoodorum* Hill, 1939 from North American mustelids. Canadian Journal of Zoology 61:2168–2178.

LANKESTER, M.W., AND R. C. ANDERSON. 1971. The route of migration and pathogenesis of *Skrjabingylus* spp. (Nematoda: Metastrongyloidea) in mustelids. Canadian Journal of Zoology 49:1283–1293.

LANKESTER, M. W., AND V. J. CHRICHTON. 1972. *Skrjabingylus lutrae* n. sp. (Nematoda: Metastrongyloidea) from otter (*Lutra canadensis*). Canadian Journal of Zoology 50:337–340. doi.org/10.1139/z72-046

LEIGH, J. W., AND D. BRYANT. 2015. Popart: full- feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116. doi.org/10.1111/2041-210X.12410

LEWIS, J. W. 1967. Observations on the skull of Mustelidae infected with the nematode, *Skrjabingylus nasicola*. Journal of Zoology (London) 153:561–564.

OERTLI, E., P. WILSON, P. HUNT, T. SIDWA, AND R. ROHDE. 2009. Epidemiology of rabies in skunks in Texas. Journal of American Veterinary Medical Association 234:616–620. 10.2460/javma.234.5.616

PETROW, A. M. 1928. Addition to the explanation of systematics of nematodes parasitic in the frontal sinus and lungs of Mustelidae. Annals of Tropical Medicine & Parasitology 22:259–264, doi: 10.1080/00034983.1928.11684574

PROSSER, S. W. K., M.G. VELARDE-AGUILAR, V. LEÓN- RÈGAGNON, AND P.D.N. HEBERT. 2013. Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. Molecular Ecology Resources 13:1108–1115. doi.org/10.1111/1755-0998.12082

RUBINOFF, D., S. CAMERON, AND K. WILL. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. Journal of Heredity 97:581–594. doi:10.1093/ jhered/esl036

SANTI, S. A., AND G. H. PARKER. 2012. Biology of sinus worm (*Skrjabingylus*) infections in mustelid species: Sinus worm (*Skrjabingylus*) infections in mustelids. Lambert Academic Publishing, Saarbrücken, Germany.

TALBOT, B., D. GARANT, S. R. PAQUETTE, J. MAINGUY, AND F. PELLETIER. 2012. Lack of genetic structure and female-specific effect of dispersal barriers in a rabies vector, the striped skunk (*Mephitis mephitis*). PLoS One 7:e49736. doi:10.1371/journal.pone.0049736.

THERON, A. 1975. Experimental research on larval evolution of *Skrjabingylus nasicola* (Nematoda Metastrongyloidea) in 2 terrestrial molluscs. Vie et Milieu Serie C-Biologie Terrestre 25:49–54.

TSANG, W. Y. AND B. D. LEMIRE. 2002. Stable heteroplasmy but differential inheritance of a large mitochondrial DNA deletion in nematodes. Biochemistry and Cell Biology 80:645–654.

TUMLISON, R., AND T. L. TUMLISON. 2019. Occurrence of the sinus nematode *Skrjabingylus* sp. (Nematoda: Metastrongyloidea) inferred from sinus lesions in Arkansas Mustelidae and Mephitidae, with review of relevant literature. Journal of the Arkansas Academy of Science 73:6–12.

WEBSTER, W. A. 1965. *Skrjabingylus magnus* n. sp. (Nematoda: Trichostrongylidae) from the skunk, *Mephitis mephitis*. Canadian Journal of Zoology 43:229–231.

ZHANG, D. X. AND G. M. HEWITT. 1997. Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. Biochemical Systematics and Ecology 25:99–120.

ZHOU, X., Y. XIE, Z. ZHANG, C. WANG, Y. SUN, X. GU, S. WANG, X. PENG, AND G. YANG. 2013. Analysis of the genetic diversity of the nematode parasite *Baylisascaris schreoderi* from wild giant pandas in different mountain ranges in China. Parasites and Vectors 6:233. doi.org/10.1186/1756-3305-6-233

APPENDIX I- Listed are the 39 COI sequences for *Skrjabingylus chitwoodorum* included in the analysis. Sequences are listed in the same order as seen in Table 1 with the corresponding ASK reference number and county of host origin.

ASK12134 Clinton
AGTTGTCTAAGCCAGGGATGCTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGTTACCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACACTAGGTCATCCTGGTAGGAGGGTGGATTTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK12130 Franklin
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGTTACCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACACTAGGTCATCCTGGTAGGAGGGTGGATTTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK12139 Franklin
AGTTGTCTAAGCCAGGGATGCTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGTTACCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACACTAGGTCATCCTGGTAGAAGGGTGGATTTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK11870 Brule
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGTTACCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACACTAGGTCATCCTGGTAGGAGGGTGGATTTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK14307 Brule
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTCGG
ATTCTTGTTTTGCTGATACGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTACGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGCTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGCGTACTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK14311 Brule
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATACGGGTTGTGGTACTAGATGAACTGTTTCCCCCCCGTTG
AGTACATTACGTCATCCTGGCAGAAGGGTGGATCTAGCTATTTTTAGTCTGCATT
GTGCTGGTATAAGCTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTACTTCAACTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTA
TTACTACTGTTCTTTTGTTGGTTTTATCTTCACCTGTTTTAGCTGGGG
ASK9931 Bailey
AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK9844 Bastrop
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTTATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK9752 Bee
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAAATATAAGGTTTCC

TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13294 Bell

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13265 Brown

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGTTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTAGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK12039 Collin

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGT
TATTACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTG
GTGGGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTT
TCCTCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTT
TGGATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCG
TTGAGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGC
ATTGTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAA
AATATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGG
TTTTTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9908 Collin

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA

TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
ASK9790 Colorado
AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK9877 Denton
NNNNNNNNNNNNNNNNNTGTTGTTATCTAATGGACAATTATATAATGCGGTTA
TTACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGT
GGGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTC
CTCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTG
GATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTT
GAGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCAT
TGTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAA
ATATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTT
TTTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK9913 Denton
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATGTGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTGCCCGTTTTNNNNNNNN
ASK13296 Ellis
AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG
ASK13295 Fannin
AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT

TACGGCTCATGCTATTTTGATGATTTTTTTTATGGGGATGCCTACTTTGATTGGGG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGGGCTCCAAATATAAGGTTCCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGGGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGGGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGGGGTATTAATTTTATGTGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTGCCTGTTTTAGCTGGGG

ASK9749 Fayette

AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAAATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTCTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13596 Freestone

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13245 Hays

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13244 Hood

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG

AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13619 Hutchinson

AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGGG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGGGCTCCAAATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK7426 Hutchinson

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGGGCTCCAAATATAAGGTTCCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13593 Llano

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TTCGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATGTGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTGCCTGTTTTAGCTGGGG

ASK13618 Llano

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9770 Llano

AGTTGTCTAACCCTGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGGG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTCGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

ASK9779 Llano

AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGGTGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9975 Mason

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9868 McLennan

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13275 Potter

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC

TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9774 Potter

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACCGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK13208 Reeves

AGTTGTCTAAGCCATGGATGCTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGCG
GGTTTGGGAATTGGATGTTACCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAATTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTATCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTNNNNNNNNNNNNNNNNN

ASK9865 Sutton

AGTTGTCTAAGCCAGGGAAGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGATTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTACGTCCTCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCCTTCCCTGTTTTAGCTGGGG

ASK13259 Tarrant

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA

TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9750 Travis

AGTTGTCTAACCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAAATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCTCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATGTGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTGCCTGTTTTAGCTGGGG

ASK13278 Webb

AGTTGTCTAAGCCAGGGATGTTGTTATCTAATGGACAATTATATAATGCGGTTAT
TACGGCTCATGCTATTTTGATGATTTTTTTTATGGTGATGCCTACTTTGATTGGTG
GGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAGATATAAGGTTTCC
TCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTGG
ATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTTG
AGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCATT
GTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAAA
TATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTTT
TTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTANNNNNNN

ASK9784 Williamson

NNNNNNNNNNNNNNNNNNGTTGTTATCTAATGAACAATTATATAATGCGGTTA
TTACGGCTCATGCTATTTTGATGATTTTTTTTTATGGTGATCCCTACTTTGATGGGG
GGGTTGGGGAATTGGATGCTGCCTTTAATATTGGGTGCTCCAGATATAAGGTTTC
CTCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGGTTTTG
GATTCTTGTTTTGTTGATATGGGTTGTGGTACTAGATGAACTGTTTACCCCCCGTT
GAGTACATTAGGTCATCCCGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCAT
TGTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAA
ATATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTT
TTTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

ASK9746 Williamson

NNNNNNNNNNNNNNNNNNNNNNNTATCTAATGGACAATTATATAATGCGGTT
ATTACGGCTCATGCTATTTTGATGATTTTTTTTATGGGGATGCCTACTTTGATTGG
GGGGTTTGGGAATTGGATGCTGCCTTTGATATTGGGTGCTCCAAAAATAAGGTTT
CCTCGTTTAAATAATTTAAGTTTTTGGTTGTTGCCAACAGCTATGTTTTTGATTTT
GGATTCTTGTTTTGTTGATATGGGTTGTGGTACTAAATGAACTGTTTACCCCCCGT
TGAGTACATTAGGTCATCCTGGTAGAAGGGTGGATCTAGCTATTTTTAGTTTGCA
TTGTGCTGGTATAAGTTCAATTTTGGGTGGTATTAATTTTATATGTACTACTAAAA
ATATGCGGAGTAGTTCAATTTCTTTGGAGCATATAAGTTTATTTGTTTGAACGGTT
TTTATTACTGTTTTTTTGTTGGTTTTATCTTTACCTGTTTTAGCTGGGG

BIOGRAPHY

Allie Neila Denham was born on March 3rd, 1998 in San Antonio, Texas, to two loving parents, Richard Denham and Jacqueline Innanen. Allie excelled in sports throughout high school and initially attended McMurry University to pole vault and study Biomedical Science on scholarship. Allie decided to transfer to Angelo State University and join the ASU Honors Program to continue her academics as she saw herself growing more interested in genetics rather than medicine. Allie became involved in the Honors Student Association as Historian and later on President, as well as becoming a member Tri-Beta. During the spring semester of junior year Allie became employed within the Angelo State Natural History Collections that ultimately led her to this work for an Honors thesis on *Skrjabingylus*. She has presented her work multiple times and has won awards such as the Clyde Jones Undergraduate Poster Award in molecular systematics at the Texas Society of Mammalogists conference. Allie graduated from ASU with a Bachelor of Science in Biology and Highest University Honors in May of 2020. Despite the Coronavirus impacting her senior year, Allie will attend Texas A&M University in the fall where she has accepted a fellowship in the Ph.D. GENE Program in Interdisciplinary Genetics.