The Dissertation Committee for Stavana Elaine Strutz Certifies that this is the approved version of the following dissertation:

Cutaneous Leishmaniasis Emergence in Texas: Changing Patterns of Disease and Hosts

Committee:

Camille Parmesan, Supervisor

Steve Phelps, Co-supervisor

Tim Keitt

Craig R. Linder

Lauren Meyers

Jennifer Miller

Cutaneous Leishmaniasis Emergence in Texas: Changing Patterns of Disease and Hosts

by

Stavana Elaine Strutz

Dissertation

Presented to the Faculty of the Graduate School of Integrative Biology

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December 2017

Dedication

To the strongest women I know: my mother, Ulana Ratley, and my sister, Tammy Dettmann.

Acknowledgements

I want to thank my family for their continued support, especially my mother, Ulana Ratley, and my sister, Tammy Dettmann, for countless hours of encouragement. I also want to thank those family members that supported my research by helping me with my field work, my grandmother, Joreen Ludeke, and my uncle, Max Ludeke.

I especially wish to acknowledge my advisors, Dr. Camille Parmesan and Dr. Steve Phelps! Without their continued support, constructive criticism, and pep talks, this dissertation would not have been achieved. I want to thank my committee for taking the time to review my work each year and give me helpful feedback: Dr. Tim Keitt, Dr. Randy Linder, Dr. Lauren Meyers, and Dr. Jennifer Miller. This research would have been impossible without the assistance and support of my French colleagues: Dr. Jerome Depaquit, Dr. Leila Hadj Henni, Dr. Denis Augot, and the undergraduate students at the University of Reims, France.

Multiple faculty and administrative staff have been very important to my success: Dr. Ed Theriot, Dr. Pamela Owen, Dr. Ophelia Wang, Dr. Claus Wilke, Tamra Rogers, Sylvia Moore, Theresa Kelly, Frances Lemear, Valerie Croft, Sandy Monahan, Sean Schaffer, Dr. Thomas E. Lindsey, and Dr. Sara Saylor.

Without my undergraduate research assistants, this dissertation would have been impossible. So a big thank you to: Cory Schlesener, Ryan Baggerly, Douglas McDowell, Emily Seltzer, Brenda Johnson, Eric Arbour, Alex Fazzone, Meredith Shae Suggs, Marcos Rivera, Shawn Ling, Sergio Aleman, Andrew Hartford, Andrew Wang, Brenda Johnson, Lauren Beach, Maria Espinosa, Jose Guevara, Kira Holland, Emma Hines, Ina Grose, Danielle Moore, and Joseph Garcia.

I also must thank the various individuals working at the National Institutes of Health, the Centers of Disease Control, and the Texas Department of State Health Services for their assistance: Dr. Jesus Valenzuela, Dr. Shaden Kamhawi, Dr. Shannon Townsend, Dr. Francis Steurer, Dr. Marcos de Almeida, Dr. Jim Schuermann, Dr. Tom Sidwa, Nicole Evert, Cathy Snider, Inger Vilcins, and Guy Moore.

Finally, I must thank my friends who have stood by me through this entire process and given me their continued love and support, Dan Warren, Jessica Lynn Davis, Jacob Walden Soule, Julie Laursen, Mariska Brady, Caitlin McClune, Shalmali Joshi, Kendra Cook, Jason Ikpatt, Mariam Okhovat, Alejandro Berrio, Teresa Iglesias, Yuriana Kimmons, Robert Ellis, Mark Duncan, Karen Duncan, Brady Duggan, Tamara Teigan, Audrey Nha Lan Nguyen, Kitty May Calhoun, Rowan Calhoun, David Zheng, Laura Takakjy, Calla Hummel, Toni Golan-Villa, Erin Giglio, Lurko Pastrana, Maggie Meadows, and Vicky Huang. I also must thank those that have given me guidance: Dr. Katherine Rees, Dr. Mark Zentner, Dr. Mona Ghosheh, and Rahel Pearson.

My final shoutout goes to my pets for all their cuddles during the difficult times: Zero the dog, Zeka “Fluffikins” Strutz, Contessa “Bad Kitty” Strutz, and Charlie the destroyer.

Cutaneous Leishmaniasis Emergence in Texas: Changing Patterns of Disease and Hosts

Stavana Elaine Strutz, PhD

The University of Texas at Austin, 2017

Supervisor: Camille Parmesan

Co-supervisor: Steve Phelps

Cutaneous leishmaniasis, a vector-borne protozoan disease caused by *Leishmania mexicana*, has emerged in northeastern Texas and Oklahoma. An exploration of the literature was carried out to find all documented cases of the disease or parasite. An exhaustive list of potential vectors (species of phlebotomine flies), reservoirs (woodrat species of the genus *Neotoma*), and other hosts was created based on World Health Organization criteria. A phlebotomine species, *Psathromyia shannoni*, was identified as a new potential vector based on WHO criteria and its distributional overlap with new cases. Species distribution models of *Le. mexicana* occurrences or cutaneous leishmaniasis cases were used to construct species distribution models between two periods of disease activity: 1982-1994, and 2001-2015. A northeastern expansion and range shift was predicted when recent cases were projected to the past; however, when SDMs calibrated on past data were projected into the future, a weaker range shift was predicted. In order to obtain more accurate disease occurrence data, potential vectors and reservoirs were broadly sampled across Texas with an emphasis on phlebotomine flies and rodents. Seven species of phlebotomines were identified via morphological and molecular methods. A barcoding analysis was carried out by examining the *cyt b* gene. Surprisingly, four separate

morphologically identified species of the genus *Micropygomyia* grouped together on a single clade suggesting that they may actually comprise a single species. Multiple species were documented beyond their original range, namely one of the suspected vectors, *Dampfomyia anthophora*. Identified phlebotomines were screened for *Le. mexicana* by site and species using PCR. The majority of geographical sites tested negative for the parasite. However, a single *D. anthophora* fly at an Ecolab site located in Brazos County tested positive. Subsequent testing of the woodrat, *Neotoma floridana*, and White-footed mouse, *Peromyscus leucopus*, at the same site were negative. Only recently has *Le. mexicana* been documented in the eastern woodrat suggesting a species jump may have occurred. It is important to note that the parasite has for the first time been documented at a site where both the vector, *D. anthophora*, and the eastern woodrat occur.

Table of Contents

List of Tables

List of Figures

Chapter 1: Cutaneous leishmaniasis in the southern United States

ABSTRACT

Cutaneous leishmaniasis caused by *Leishmania mexicana* has emerged in the southern United States well beyond its historical range. Multiple causative factors may be contributing to the expansion: climate change, host species jumps, sylvatic habitat encroachment by humans, sampling bias, and/or reporting bias. To date, there has not been a large, systematic review of the vectors, reservoirs, and incidental hosts in the southern United States thus making it difficult to discern what is driving the emergence. In order to analyze why the geographic range of cutaneous leishmaniasis is changing, an examination of vector and reservoir classification was carried out using World Health Organization criteria. A new species of phlebotomine fly, *Psathromyia shannoni,* was found to meet 4 out of 5 WHO criteria thus making it a potential vector in regions of emergence. It had never before been implicated. This study also combined data from the literature and the Texas Department of State Health Services to accurately assess the number and location of infections. New human infections were documented in northern, central, western, and eastern regions of Texas but not in south Texas. Cases found in non-human hosts were also aggregated and presented from the literature. This analysis represents the first step in an increased understanding of the eco-epidemiology of cutaneous leishmaniasis in the southern United States.

INTRODUCTION

Anthropogenic climate change has been linked to altitudinal and latitudinal shifts across taxa (Hickling et al., 2006; Parmesan, 2006; Perry et al., 2005; Poloczanska et al., 2013). Multiple global meta-analyses have found terrestrial species to have shifted 6-11 meters upwards in altitude per decade and 6.1-17.2 km further poleward per decade (Chen et al., 2011; Parmesan and Yohe, 2003) while leading range edges of marine species have moved 72 km per decade in the expected poleward direction (Poloczanska et al., 2013). Given that large groups of terrestrial and marine biota are shifting their geographical

distributions due to climate change, parasites and their carriers are also likely to be undergoing similar geographical changes. Many scientists have predicted that climate change will alter geographical distributions of various diseases and their carriers (Freed et al., 2005; Genchi et al., 2011; Gonzales et al., 2010; Mills et al., 2010; Nakazawa et al., 2007; Ostfeld, 2009; Polley and Thompson, 2009).

So far, data are scarce, but a few recent studies suggest that certain vector-borne diseases are emerging as a consequence of climate change. A disease is said to be emerging when there is a surge in incidence, appearance in a new population, or expansion of the geographical range (Morse, 1995). Rosenthal et al. (2015) added additional definitions of disease emergence: when a disease increases in impact, when a pathogen has undergone recent evolutionary change, when a pathogen is detected in the human population for the first time, when a pathogen significantly changes its pathology or clinical presentation, or when a pathogen is discovered for the first time (Rosenthal et al., 2015).

A particular disease that has been shown to be very sensitive to shifts in temperature and precipitation and thus likely to be influenced by climate change and become an emergent disease is leishmaniasis. Multiple studies have found leishmaniasis incidence to be related to variability in annual climatic and other environmental variables (Ali-Akbarpour et al., 2012; Cardenas et al., 2006, 2006; Chaves and Pascual, 2006; Cross and Hyams, 1996; Elnaiem et al., 2003; Franco et al., 2011; Franke et al., 2002; Thompson et al., 2002; Thomson et al., 1999; Toumi et al., 2012). One area where it is likely to be emergent is the southern United States.

Leshmaniasis is caused by parasites of the genus *Leishmania* and is considered the second deadliest human parasitic disease behind malaria. It is transmitted by phlebotomine fly vectors and carried by various mammalian reservoirs. Biological vectors spread parasites while animal reservoirs serve as localities of parasite development and amplification by remaining infected for extended periods. Over 350 million people are considered to be at risk of leishmaniasis and it is found in 98 countries across the world (World Health Organization, 2010). 2 million people are infected annually and approximately 50,000 of those infections result in death (World Health Organization,

2010). Leishmaniasis is considered a neglected tropical disease although it is found throughout tropical, subtropical, and temperate zones and is also categorized as an emerging and re-emerging pathogen (Ashford, 2000).

LIFE HISTORY

There are 17 known species of *Leishmania* that cause disease in humans (World Health Organization, 2010) although there are efforts to change the taxonomy and reduce the number of species (Schönian et al., 2010). Leishmania parasites are classified in the *Trypanosomatidae* family along with the parasites that cause Chagas disease and African sleeping sickness. Leishmaniasis is spread by sanguivorous phlebotomine flies among mammalian and reptilian hosts.

The parasite has two stages in its life cycle: a flagellated mobile and potentially sexual cell type (promastigote) in the sand fly vector, and an unflagellated clonal cell type (amastigote) in the mammalian host (Reithinger et al., 2007). A female sand fly becomes infected with amastigotes when it feeds from an infected reservoir host (Bates, 2007). The amastigotes replicate into promastigotes in the fly's gut and migrate to the salivary glands (Kamhawi, 2006). At this time the parasite sexually reproduces inside the phlebotomine fly vector (Rougeron et al., 2009, 2010, 2011). In order to be categorized as a vector or reservoir, a species must meet certain criteria. The female phlebotomine fly eventually feeds again and passes the mobile promastigotes into the next host (Desjeux, 2004) or reservoir. Promastigotes are phagocytized by white blood cells that become Trojan horses for discreet clonal replication of the parasite allowing the cycle to begin anew. These pathogens are particularly difficult to treat due to eukaryotic similarity to the host and their preferred invasion of immune cells like macrophages.

While all leishmaniasis parasites follow this same basic disease transmission pattern, the different species and sometimes even the same species of parasites produce different disease manifestations. Visceral leishmaniasis (VL) is the most deadly form of the disease and infects inner organs such as the spleen, liver, and bone marrow. If untreated, VL can be fatal. Cutaneous leishmaniasis (CL) remains localized to the epidermis at the

site of the infective sand fly bite causing a pustule which will develop into a crater-like lesion. Diffuse cutaneous leishmaniasis (DCL) produces lesions like CL but the lesions appear in various locations on the body. This form is commonly associated with immune disorders. Mucocutaneous leishmaniasis (MCL), a particular subtype of CL, infects not only the skin but also the mucous membranes, especially those of the nose and mouth. This particular type of leishmaniasis is more dangerous and debilitating than CL or DCL as it is prone to cause secondary infections and disfigurement as the immune system destroys tissue. Scarring is much more pronounced and can lead to destruction of nasal and lip tissue.

BIOGEOGRAPHY

Cutaneous leishmaniasis in the Americas is found mainly within Mexico, Central America, and South America with a small focus in the southern United States in south Texas. During the past four decades, cutaneous leishmaniasis has appeared more than 560 km northeastward of its original range in southern Texas.

While cutaneous leishmaniasis in northern Mexico and the southern United States has been mainly associated with prickly pear and mesquite scrublands (Ashford, 2000), within the rest of Central America cutaneous leishmaniasis is primarily a disease of hardwood forests (Disney, 1968). The parasite's recent appearance in the Blackland Prairie and Oak Wood regions of Central and North Texas should not be dismissed. If the parasite has expanded its geographic range via already implicated vectors and reservoirs to wooded areas in the United States, this may indeed pose a significant risk as the pathogen may be encountering a more hospitable habitat in eastern portions of the United States. Additionally, a new enzootic cycle may have begun in northern and eastern Texas in new hosts. The parasite was recently documented in east Texas, near the Piney Woods ecoregion (McHugh et al., 2003).

The cutaneous manifestation of leishmaniasis that occurs in Texas is caused by *Leishmania mexicana*. It is vectored by sanguivorous phlebotomine flies from several genera and spread between woodrat reservoir hosts of the genus *Neotoma* (Kerr et al., 1995,

1999; McHugh et al., 1990, 1993, 2001, 2003). Other sylvatic mammalian species have recently been implicated in the enzootic cycle of the disease.

Multiple factors may be contributing to the occurrence of the pathogen in previously unrecorded areas such as climate change, host species jumps, human expansion into sylvatic habitat, and sampling/reporting bias. Climate change is expected to have multiple effects on pathogenic organisms—one being the geographic expansion of disease as vectors and reservoirs shift their ranges to higher latitudes. The recent findings of other infected species not previously considered to be reservoirs opens the door on the question of whether a vector or reservoir species jump may have occurred. Texas has recently experienced population growth and because of that, extensive development. These changes in population may be contributing to contact between humans and wild species that had not previously occurred. Additionally, because leishmaniasis was not made reportable until 2007, sampling bias may be playing a role as well as reporting bias from medical professionals.

The goals of this study were to analyze the effects of climate change on an emerging pathogen, cutaneous leishmaniasis, to determine whether or not the geographical ranges of disease are responding to changes in climatic variables. The dynamics of leishmaniasis ecology make it an excellent study system due to the recent emergence of the pathogen in various parts of the world. In order to examine such an emergence in the southern United States, a comprehensive literature review of known cases, a broad sampling of vectors and reservoirs, and extensive screening for the pathogen were carried out. This chapter consists of a literature review that involved gathering data from the Texas Department of State Health Services, and an analysis of the vector, reservoir, or host status of all organisms infected to date.

PHLEBOTOMINE FLY VECTORS

In order to categorize a sand fly as an anthroponotic vector (vector of disease in humans), the species must meet several criteria according to the World Health Organization (WHO): (1) the vector must be anthropophilic; (2) the vector must bite the reservoir host(s);

(3) the vector must be infected with the same species of *Leishmania* as that infecting humans; (4) the vector must support the growth and maturation of the parasite; (5) the vector must be able to transmit the parasite by bite (World Health Organization, 2010). Most species of sand flies in Texas suspected of transmitting leishmaniasis have not yet fully met the stringent WHO criteria to be definitively considered vectors of human cutaneous leishmaniasis, though some can be categorized as enzootic or zoonotic vectors. Other classification schema exist (Killick-Kendrick, 1990) but the World Health Organization criteria is universally used by *Leishmania* experts for categorization. Additionally, it is unclear if the disease cycle in the Southern United States consists of the same host assemblages. It is difficult to speculate on vector status when there may be different geographical foci of the same parasite. Still, several species in the United States partially meet vector criteria (Table 1.1).

Sand fly species	***Location***	***WHO Vector Criteria***	***Suspected Host Preference***	***References***
D. anthophora	Mexico, AZ, TX	2, 3, 4, 5	woodrat (*N. micropus*)	(McHugh et al., 1993; Mead and Cupp, 1995; Perkins et al., 1987a; Young and Perkins, 1984)
Lu. cruciata	Panama to SE Mexico, FL, GA	1, 4, 5	human	(Williams, 1966; Young and Perkins, 1984)
Lu. diabolica	SW Mexico to TX	1, 4, 5	human	(Lawyer and Young, 1987; Lawyer et al., 1987; Young and Perkins, 1984)
Ps. shannoni	Argentina to USA, AL, AR, DE, FL, GA, KS, KY, LA, MD, MS, MO, NC, OH, SC, TN, TX	1, 3, 4, 5	human, sloth, some birds	(Christensen and de Vasquez, 1982; Claborn et al., 2009; Lawyer and Young, 1987; Lawyer et al., 1987; Minter et al., 2009; Pech-May et al., 2010; Weng et al., 2012; Young and Perkins, 1984)

Table 1.1: Table of phlebotomine fly species found in the United States that meet some vector criteria of the WHO.

Recently the genus *Lutzomyia* has undergone taxonomic change and been split into several genera; I will use the more recent systematic classification system constructed by Galati (Shimabukuro et al., 2017).

One potential enzootic vector, *D. anthophora,* is found ranging from Mexico to the southwestern United States in woodrat nests and near unidentified rodent burrows (Young and Perkins, 1984). Thus far, *D. anthophora* seems the most likely vector and at least can be said to be an enzootic vector even though it fails the first criterion (Table 1.1). *D. anthophora* was found infected with *Le. mexicana* in southeastern San Antonio (Figure 1.1), Bexar county, Texas (McHugh et al., 1993) and has also successfully transmitted the parasite to hamsters in a lab setting (Perkins et al., 1987b). This particular species of sand fly is a nest associate with *N. micropus*, the Southern Plains Woodrat, which is the only verified rodent reservoir thus far (Grogl et al., 1991; McHugh et al., 1990; Young, 1972). One bite from an infected sand fly was found to be sufficient to transmit *Le. mexicana* (Perkins et al., 1987b) to hamsters.

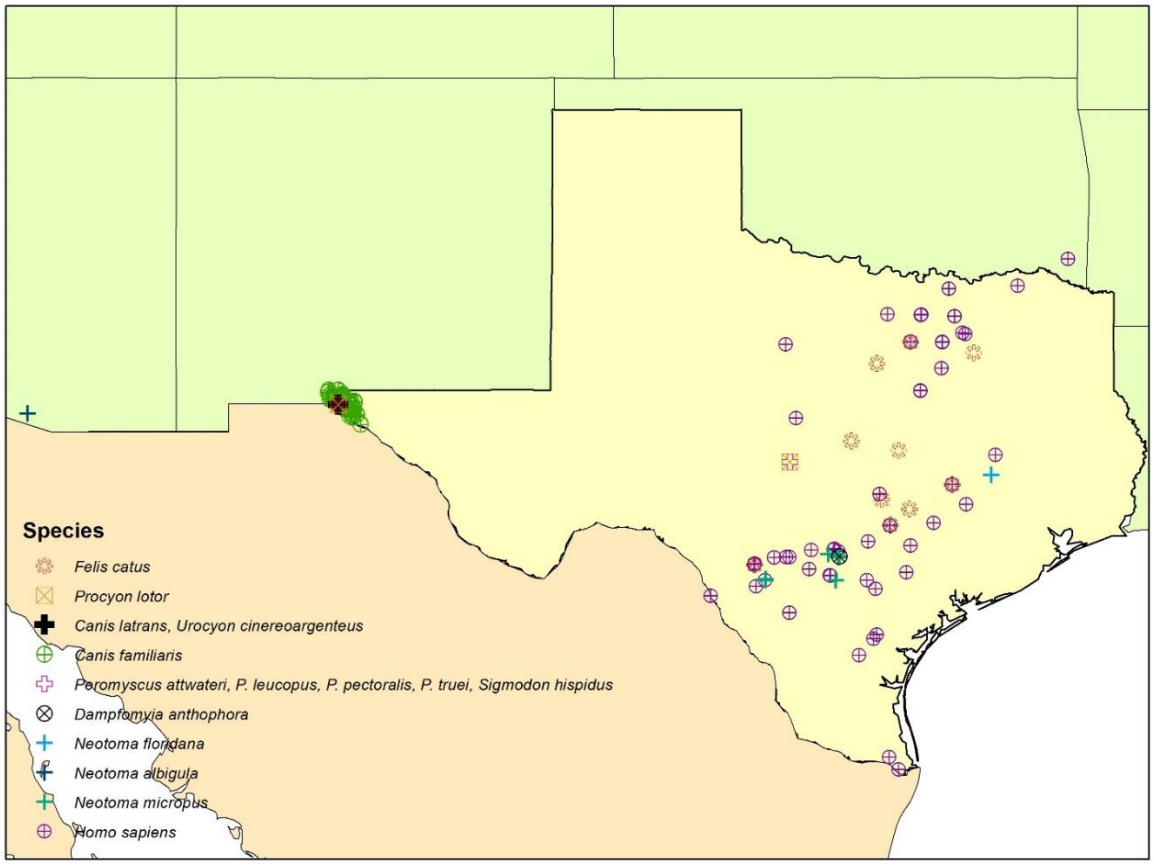

Figure 1.1: Multiple species have been infected with *Le. mexicana* or shown symptoms of cutaneous leishmaniasis in Arizona, Texas, and Oklahoma. These species represent

phlebotomine flies (*D. anthophora),* companion animals such as a cats and dogs, and sylvatic mammals like rodents and various canines.

Two other species, *Lu. diabolica* and *Ps. shannoni,* have been suggested as possible vectors (World Health Organization, 2010); however, *Ps. shannoni* has not been suggested as a vector in the Texas foci of leishmaniasis. In experimental laboratory studies, *Le. mexicana* amastigotes were able to undergo division and maturation into highly mobile promastigotes within these phlebotomine fly species (Lawyer et al., 1987). Additionally, another study found that these species were both capable of transmitting the parasite to hamsters (Lawyer and Young, 1987). Both species are anthropophilic feeders and may serve as a bridge between humans and enzootic reservoir hosts (Lawyer and Young, 1987).

Additionally, *Ps. shannoni* was found infected with *Le. mexicana* in the Yucatan Peninsula of Mexico (Pech-May et al., 2010). This particular species of sand fly fulfills four of five WHO vector criteria making it a likely vector of leishmaniasis (Table 1). *Ps. shannoni* is also associated with hardwood forest habitats and may actually be undergoing a range expansion (Weng et al., 2012). It was thought to be a probable vector of leishmaniasis in Costa Rica as well, although for *L. braziliensis* (Zeledón and Alfaro, 1973). Cases of cutaneous leishmaniasis have begun to appear in eastern Texas and overlap with the range of *Ps. shannoni* suggesting that this species may be a vector within the United States.

Lu. cruciata is closely related to *Lu. diabolica* and very difficult to discriminate from the latter in areas of their shared range, especially in Mexico and south of Mexico. *Lu. cruciata* was found infected with *Le. mexicana* in Mexico and Honduras (Pech-May et al., 2010; Williams, 1966). This species also enthusiastically feeds on humans and was found to transmit cutaneous leishmaniasis to human study participants in an early experiment (Williams, 1966). Three out of five WHO vector criteria are fulfilled with the fourth being implied as the parasite can mature within the sand fly. This makes *Lu. cruciata* another likely cutaneous leishmaniasis vector; however, not in Texas as the only known species occurrences in the United States are in Florida and Georgia (Young and Perkins, 1984).

Other species of phlebotomine fly occur in the United States such as *M. apache, D. aquilonia, M. californica, M. oppidana, M. stewarti, Lu. tanyopsis, Ps. texana, Pi. xerophila,* and *M. vexator* with varying vector classification and blood meal host preferences. These species have not yet been implicated in the enzootic or anthroponotic cycles of leishmaniasis.

MAMMALIAN RESERVOIR HOSTS

Reservoirs are animal hosts where parasite development and amplification occurs, generally without causing severe morbidity to the host. There are several different definitions of what constitutes a reservoir host but the definition from the WHO will be used in this review (World Health Organization, 2010). Reservoir hosts exist in several categories. The principal reservoir host is the most important because it maintains the parasite in the ecological system. Minor or secondary reservoir hosts play a secondary role in maintaining the parasite. Incidental hosts play no role at all in maintaining the parasite within the ecological system (World Health Organization, 2010). Finally, liaison hosts transmit the parasite from animals to humans. This last concept comes from Ashford and is not found in the WHO Report (Ashford, 1996).

The WHO criteria for a principal reservoir host are less clear than the criteria for vectors and require large amounts of ecological evidence. The WHO identifies principal reservoir hosts as having the following characteristics: (1) an abundant and long-lived population that can provide a consistent food source for sand flies; (2) intense host-sand fly contact making transmission likely between different individuals of a host population; (3) high proportions of individuals within a host population infected during their lifetime; (4) infection is long lasting and nonpathogenic so that parasites can survive non-transmission seasons; (5) parasites are available in the skin or blood in sufficient numbers for vector infection. The WHO criteria do not explicitly say all criteria must be met in order for a species to be considered a reservoir.

A review of reservoirs by Ashford found that New World principal reservoir hosts may fail some portions of these criteria related to long life and close association with

vectors. He mentions that species abundance may be the most important factor. Multiple mammalian species have been found infected with leishmaniasis in the southern United States and Central America. However, the reservoir status of many of these species is unknown. This is especially true if, as Ashford suggests, most of the WHO reservoir criteria are unnecessary in New World foci of the disease (Ashford, 1996). New World reservoirs seem to only meet the criterion of abundance, criterion 1. This may be an artifact of poor research into rodents in the New World.

The Southern Plains Woodrat, *N. micropus*, is considered the principle reservoir host in Texas. Some of the first clues that *N. micropus* was the reservoir came from the observation that the range of human cases fell within the range of the Southern Plains Woodrat (Merkelz and Kerr, 2002). The Southern Plains Woodrat's range extends from northern Mexico through the southwestern region of the United States into Texas, New Mexico, Arizona, and even as far north as Kansas (Schmidly, 2004). Within Texas, they build nests of sticks and underground burrows in prickly pear and mesquite brushland (Merkelz and Kerr, 2002).

The burrows provide a humid microenvironment perfect for phlebotomine flies. In fact, *D. anthophora* is considered an inquiline resident and found at high numbers within woodrat burrows. *Le. mexicana* has been detected in in this woodrat species in 6 separate locations across the state of Texas (Table 1.2) (Kerr et al., 1995; McHugh et al., 1993; Raymond et al., 2003). The Southern Plains Woodrat lives up to 27 months in the wild at densities ranging from 1.5-50 woodrats per hectare (Merkelz and Kerr, 2002; Raun, 1966). Leishmaniasis prevalence rates of up to 27% were documented within a population of this woodrat species (Kerr et al., 1995). Simulation studies carried out by Kerr et al. also support the categorization of *N. micropus* as a principal reservoir (Kerr et al., 1997). *N. micropus* thus satisfies the first, second, and third criteria of the World Health Organization.

SPECIES	NUMBER	STATE	COUNTY	REFERENCE
Canis latrans	4	Texas	El Paso	(Matamoros, 2016)
Neotoma albigula	6	Arizona	Pima	(Kerr et al., 1999)
Neotoma floridana	1	Texas	Grimes	(Raymond et al., 2003)
Neotoma micropus	2	Texas	Atascosa	(Kerr et al., 1995)
Neotoma micropus	6	Texas	Zavala	(Kerr et al., 1995)
Neotoma micropus	18	Texas	Bexar	(Kerr et al., 1995; Raymond et al., 2003)
Neotoma micropus	22	Texas	Bexar	(Raymond et al., 2003)
Peromyscus leucopus	3	Texas	Mason	(Mariscal, 2013)
Peromyscus pectoralis	1	Texas	Mason	(Mariscal, 2013)
Peromyscus truei	1	Texas	Mason	(Mariscal, 2013)
Peromyscus attwateri	2	Texas	Mason	(Mariscal, 2013)
Procyon lotor	10	Texas	El Paso	(Matamoros, 2016)
Procyon lotor	3	Texas	Mason	(Mariscal, 2013)
Sigmodon hispidus	2	Texas	Mason	(Mariscal, 2013)
Urocyon cinereoargenteus	4	Texas	El Paso	(Matamoros, 2016)

Table 1.2: Sylvatic mammals found infected with *Le. mexicana* or diagnosed with cutaneous leishmaniasis in Texas.

N. floridana, the Eastern Woodrat, has also been found infected in the eastern-most isolation of the disease (Table 1.2). The Eastern Woodrat is a larger and browner woodrat than the Southern Plains Woodrat preferring wooded habitat. It builds its nests using sticks

in burrows and trees, and its range extends from Texas in the southwestern United States to South Dakota in the North, Florida in the Southeast, and North Carolina in the Northeast. A male woodrat was found in Grimes County, Texas, with scabbed nodular and cauliflower-like swellings of the pinnae of the ear and swollen, lesion covered feet. The woodrat tested positive for *Le. mexicana* via PCR (McHugh et al., 2003). The occurrence of leishmaniasis in eastern portions of Texas may represent a novel focus of the disease due to recent expansion or an endemic and previously undetected ecological system. It is unclear whether this species could be a reservoir as it is actually suffering from a symptomatic infection that would likely decrease its lifespan. It does not meet the WHO criteria either.

Another southern mammal and potential principal reservoir host is the White-Throated Woodrat (*N. albigula)* in Arizona. Twenty-eight White-Throated Woodrats were found infected (Table 1.2) (Kerr et al., 1999). Additionally, *D. anthophora,* the likely enzootic vector of Texas cutaneous leishmaniasis, has been found as a nest co-inhabitant of the White Throated Woodrat (Mead and Cupp, 1995). Recently this species underwent a taxonomic split based on genetic differences therefore it is unclear whether or not *N. leucodon* may considered to have been infected (Edwards and Bradley, 2002; Edwards et al., 2001). East of the Rio Grande River, the species has been renamed the White Toothed Woodrat, *N. leucodon.* So far no ecological, behavioral, or morphological differences of this new species have been described from that of *N. albigula.* It is likely that this newly defined species could also be a potential reservoir of the disease.

Other rodents have also been found infected with *Le. mexicana* in Texas such as the Texas mouse (*Peromyscus attwateri*), the White-footed mouse (*P. leucopus*), the White-ankled mouse (*P. pectoralis*), the Pinon mouse (*P. truei*), and the Cotton rat (*Sigmodon hispidus*) (Table 1.2) (Kipp et al., 2016; Mariscal, 2013). Elsewhere, other related *Peromyscus* species have been found infected such as *P. yucatanicus* in Mexico (Canto-Lara et al., 1999; Chable-Santos et al., 1995). Although these species were found infected, infection does not necessarily warrant reservoir status. In the past few decades, *S. hispidus* appears to have expanded its northern range (Cameron and Spencer, 1981;

Schmidly, 2002) and a new altitudinal record in New Mexico has been documented as well (Dunnum et al., 2002). *S. hispidus* was also found infected in Campeche, Mexico, with *Le. mexicana* (Chable-Santos et al., 1995). Although infected, this species is not thought to be a good reservoir due to its symptomatic presentation of leishmaniasis reducing its survival in the wild thus violating criterion 4 (Van Wynsberghe et al., 2009).

Rodents are not the only mammals that have been found infected: coyote, (*Canis latrans*), raccoon (*Procyon lotor*) and gray fox (*Urocyon cinereoargenteus)* (Table 1.2) (Mariscal, 2013; Matamoros, 2016) and bats (Berzunza-Cruz et al., 2015). Recently *Le. mexicana* has been documented in multiple bat species that migrate between the United States and Central and South America. Thirteen bat species were found infected in field studies exploring the prevalence of the disease in the Mexico (*Artibeus jamaicensis, A. lituratus, Carollia sowelli, Choeronsicus godmani, Dermanura phaeotis, Desmodus rotundus, Glossophaga commissarisi, G. soricina, Leptonycteris cursoae, Phyllostomus discolor, Pteronotus personatus, Sturnira lilium, S. ludovici*) (Berzunza-Cruz et al., 2015). Prevalence ranged from 4.0 to 26.9 percent for 12 of the 13 species. Future studies of bats as potential reservoirs capable of disseminating leishmaniasis in the United States are needed.

INCIDENTAL HOSTS: HUMANS AND COMPANION ANIMALS

Human cases of leishmaniasis within the United States are considered to be incidental infections. All recorded cases of cutaneous leishmaniasis in the United States are in Table 1.3. The first recorded case of potential autochthonous human cutaneous leishmaniasis in the United States can be traced back to a case from 1903 in a patient from Cameron County, Texas, that had also been in the northern states of Tamaulipas and Nuevo Leon, Mexico (Simpson MH et al., 1968). Between this case and 1979, only 3 other cases were diagnosed (Shaw et al., 1976; Stewart and Pilcher, 1945). After 1980, cases started to i and the parasite earned a new nickname, "Highway 90 Disease" based on the occurrence of the disease along the east/west highway connecting Del Rio to San Antonio. By 1996, an additional 25 autochthonous cases were described in the literature (Fumer, 1990; Golino

et al., 1991; Gustafson et al., 1985; McHugh et al., 1996; Nelson et al., 1985; Reed, 1986; Shaw et al., 1976). Most of these cases were from South Texas. However, there were two cases further north in Brown County, Texas (McHugh et al., 1996).

COUNTY	NUMBER OF CASES	REFERENCE
Atascosa	2	(McHugh et al., 1996)
Bexar	3	(Gustafson et al., 1985; McHugh et al., 1996; Nelson et al., 1985b; Reed, 1986; Vilcins, 2016a)
Brown	2	(McHugh et al., 1996)
Burleson	1	(Vilcins, 2016a)
Caldwell	2	(Vilcins, 2016a)
Cameron	1	(McHugh et al., 1996; Simpson MH et al., 1968)
Collin	4	(Clarke, 2006a; Snider, 2011; Wright et al., 2008)
Dallas	3	(Snider, 2011; Vilcins, 2016a; Wright et al., 2008)
De Witt	1	(Vilcins, 2016a)
Denton	4	(Snider, 2011; Vilcins, 2016a; Wright et al., 2008)
Ellis	2	(Snider, 2011)
Fayette	1	(Vilcins, 2016a)
Gonzales	1	(McHugh et al., 1996; Reed, 1986; Shaw et al., 1976)
Grayson	2	(Vilcins, 2016a)
Guadalupe	1	(McHugh et al., 1996; Reed, 1986)
Hill	2	(Snider, 2011; Wright et al., 2008)
Jim Wells	2	(Fumer, 1990b; McHugh et al., 1996; Reed, 1986; Stewart and Pilcher, 1945)
Karnes	2	(McHugh et al., 1996; Reed, 1986; Shaw et al., 1976)
La Salle	2	(McHugh et al., 1996)
Lamar	1	(Clarke, 2006a; Snider, 2011)
Madison	1	(Vilcins, 2016a)
Maverick	1	(McHugh et al., 1996)
McCurtain*	2	(Clarke, 2006a; Clarke et al., 2013)

Table 1.3

Medina	4	(Gustafson et al., 1985; McHugh et al., 1996; Nelson et al., 1985b; Reed, 1986)
Rockwall	2	(Snider, 2011; Vilcins, 2016a)
San Patricio	1	(McHugh et al., 1996)
Shackelford	1	(McHugh et al., 1996)
Tarrant	2	(Vilcins, 2016a; Wright et al., 2008)
Travis	2	(Snider, 2011; Vilcins, 2016a)
Uvalde	5	(Gustafson et al., 1985; McHugh et al., 1996; Nelson et al., 1985b; Reed, 1986)
Washington	1	(Maloney et al., 2002)
Wise	1	(Vilcins, 2016a)
Zavala	2	(Golino et al., 1991; McHugh et al., 1996)

Table 1.3: All known human cases of cutaneous leishmaniasis likely to have been acquired autochthonously in the United States by county and number of cases occurring in that county. All cases represent counties from Texas except McCurtain county in Oklahoma.

In 2002, another human case was diagnosed in Washington County, Texas, outside the range of the principal reservoir host (Maloney et al., 2002). This extended the range of the disease outside of the Tamaulipan Brushlands of south-central Texas and into the Post Oak Savannah and Blackland Prairies region. No case had ever been recorded so far east (McHugh, 2003). Cases soon appeared dramatically further north in Oklahoma. Four cases were identified in McCurtain County in southeastern Oklahoma starting in 2004 and through 2006 (Clarke, 2006; Clarke et al., 2013). A single *D. anthophora* female was found at one of the Oklahoma case residences (Clarke et al., 2013). South of Oklahoma a focus of the disease broke out in the Dallas/Ft. Worth metroplex and surrounding counties. *Le. mexicana* was diagnosed in nine patients beginning in 2005. These new cases in the North Texas focus fell on or just beyond the range of the principal reservoir host *N. micropus* (Wright et al., 2008). They are thought to be autochthonous.

Since this apparent expansion northeastward of the parasite, the disease has been reoccurring in central regions of the state. Twenty four locally acquired human cases have been documented by the Texas Department of State Health Services along a central corridor ranging from south to north Texas between 2007 and 2014 (Vilcins, 2016). Eight

more cases have unknown origins (Vilcins, 2016). There have so far been at least 64 human cases of leishmaniasis likely acquired in Texas up to 2014 (Table 1.3).

It is important to note that the human data collected since the 2000s does not identify where the parasite was contracted. They merely represent the county in which a resident resides. A patient may have contracted the disease within the county or not and we also do not know exactly wherein the county they are located due to HIPAA privacy restrictions. While travel history was collected regarding travel outside the state in most cases, travel history within the state was often not possible to obtain. Cases of companion animals such as cats and dogs are more likely to represent actual locations of transmission because they are less likely to travel. We have more accurate occurrence data on these species as well since there are not the same privacy restrictions. Cats, in particular, are interesting as they could possibly act as sentinels within the environment. Outdoor cats hunt and venture into rodent habitat that humans would not be exposed to.

Companion animal cases are presented in Table 1.4. It is unclear whether companion animals such as cats and dogs can serve as reservoirs or liaison hosts of leishmaniasis in our particular enzootic cycle; they are likely to be incidental hosts. The first case appeared in a cat in Uvalde, Texas, in 1984 (Barnes et al., 1993). This case is especially interesting because it is one of the few cases of diffuse cutaneous leishmaniasis, that is, leishmaniasis that occurs on multiple parts of the body. This case occurred in the same area as many of the other south Texas cases called "Highway 90 Disease." Two years later in the same region, another cat was infected with cutaneous leishmaniasis (Craig et al., 1986). After these first few appearances in south Texas, feline cutaneous leishmaniasis cases reappeared in 2004 in central Texas and then later in northern Texas (Trainor et al., 2010). Five out of eight of these relatively new Texan cases were found to be infected with *Le. mexicana* via PCR (Trainor et al., 2010).

Leishmania spp. has also been discovered in dogs. In the El Paso area, Evan Kipp collected biopsies of 159 stray dogs; 43 dogs had lesions and 41 were found to be infected with leishmaniasis via PCR (Kipp et al., 2016). Eighteen of the 41 samples found infected underwent sequencing and were found to be identical to *Leishmania mexicana.* At this

particular locality, dogs may actually be acting as reservoirs as opposed the incidental hosts.

SPECIES	NUMBER	COUNTY	REFERENCE
Canis lupus familiaris	41	El Paso	(Kipp, 2016)
Felis catus	1	Uvalde	(Barnes et al., 1993)
Felis catus	1	Uvalde	(Craig et al., 1986)
Felis catus	1	Burleson	(Trainor et al., 2010)
Felis catus	1	Caldwell	(Trainor et al., 2010)
Felis catus	1	Bell	(Trainor et al., 2010)
Felis catus	1	Hood	(Trainor et al., 2010)
Felis catus	1	Kaufman	(Trainor et al., 2010)
Felis catus	1	Lampasas	(Trainor et al., 2010)
Felis catus	1	Bastrop	(Trainor et al., 2010)
Felis catus	1	Tarrant	(Trainor et al., 2010)

Table 1.4: Known cases of cutaneous leishmaniasis in companion animals within Texas counties.

DISCUSSION

There are multiple species of phlebotomine flies and mammals that carry leishmaniasis in the United States and Mexico. These carriers may serve as vectors and reservoirs or simply be incidental hosts. In the southern United States, the ecology of phlebotomine flies, rodents, and bats as vectors and reservoirs respectively is poorly understood and understudied. Several phlebotomine fly species are likely to be enzootic vectors such as *D. anthophora, Lu. cruciata, Lu. diabolica,* and *Ps. shannoni;* although only two are currently recognized. *Lu. cruciata* is not a vector in Texas because it has a range in Central America and Georgia and Florida with a disjunction in between. If leishmaniasis ever spread to the southeastern United States, *Lu. cruciata* could possibly serve as a vector as it meets several criteria in other regions.

Mammalian species, especially in the New World, are much more difficult to identify as reservoirs, potentially due to major voids in data. Several woodrat species may be reservoirs but thus far have been shown to satisfy only two WHO criteria. Multiple other rodent, bat, canine, and sylvatic mammals have been found infected with the leishmaniasis parasite. Newly documented rodent hosts suggest that the disease may be much more widely spread than previously before thought, especially the cases in Mason Mountain Wildlife Management Area. The studies on migratory bat species that carry leishmaniasis and move between Mexico and the United States also may represent another novel means by which the parasite has been spreading northward. Additionally, coyotes and gray foxes have much larger ranges than woodrats representing yet another means of parasite dispersal. More studies are needed to discern these mammals' roles in the enzootic cycle(s) within the United States.

Leishmaniasis records are being seen further North and East than ever before in humans and companion animals. The cases in West Texas in the El Paso area may represent a different focus from those occurring in South, Central, North, and East Texas. It is currently unknown whether these foci have similar enzootic cycles due to different species being involved but it is likely as the initial reservoir, the Southern Plains Woodrat, does not occur in East Texas. These El Paso cases could also represent a centrally located documentation of the disease within its range since the pathogen has been recorded as far West as Arizona. The El Paso focus also may represent a system in which dogs serve as a primary reservoir of the disease.

The parasite was previously only documented in the Southern Plains Woodrat but now has been found in the Eastern Woodrat. This suggests that there may have been a reservoir jump that is now contributing the eastern expansion of the pathogen. Alternatively, the parasite could have shifted into a new phlebotomine fly species e.g., *Ps. shannoni*, that meets four of five WHO vector criteria, although this species has not yet been found infected.

Alternatively, the simplest explanation may be that the sampling and reporting bias has led to an apparent disease expansion. It is possible, the species has been endemic

throughout much of Texas but was underreported and/or misdiagnosed. It is possible that as Texas has grown in population and seen increases in urban sprawl, humans are more often coming into contact with the pathogen providing more opportunities for transmission between phlebotomine vectors and humans.

It is clear that more research is needed into the eco-epidemiology of leishmaniasis in the southern United States. Human studies may be limiting, however, due to HIPAA privacy restrictions. More ecological studies of potential reservoirs and vectors should be carried out among accessible areas of Texas and in coordination with private landowners. Furthermore, transmission studies of various species of phlebotomines and among various mammals should also be performed. Another possibility would be to carry out a coordinated effort between researchers and veterinarians to obtain information on disease location based on infected pets. Trapping for reservoirs and vectors within locations where the disease occurs in pets could be obtained with owner permission.

Chapter 2: Climate change and leishmaniasis

ABSTRACT

Cutaneous leishmaniasis has expanded its geographical range in the southern United States, specifically in Texas and Oklahoma, in concordance with our expectations of climate change driven latitudinal movement of species ranges toward the poles. Multiple studies have forecasted changes in geographic distributions of vector-borne diseases in the future but few have examined whether or not those changes have already occurred. Additional factors such as a host species jump, human encroachment on sylvatic habitat, sampling bias, and recording bias may have also played a role. In order to examine whether or not climate change is a driver of the recent emergence, species distribution models were constructed across time both forward and backward using precipitation and temperature variables. Both models predicted a northward shift of the parasite's range with decreases in probability of presence in south Texas where the parasite is no longer being documented. A principal component analysis (PCA) was carried out to determine whether or not the climate space or niche of the parasite had changed between time periods. The PCA showed overlap in climate space but also occupation of new climate space suggesting that the climate space had shifted geographically and that the niche of the parasite had expanded. This supports the hypotheses of both climate change driven emergence and a species jump.

INTRODUCTION

Infectious disease emergence is a serious but rarely documented consequence of climate change. Pathogens and their carriers can be released from ecological constraints in areas that experience environmental changes, for example, increased precipitation and/or hotter temperatures. Hotter temperatures can increase replication, survival, and development of pathogens and the feeding rate of sanguivorous (blood-sucking) vectors; increases in precipitation enlarge arthropod vector and rodent reservoir populations and extend their breeding seasons (Harvell et al., 2002; Ostfeld, 2009; Parmesan, 2006; Patz and Olson, 2006). Additionally, disease emergence is predicted to be enhanced by host

range growth, particularly shifts toward higher latitudes, and upward shifts in altitude (Parmesan, 2006).

There are multiple causes of disease emergence. One occurs when range changes bring potential host populations in direct proximity to the parasite. This may be due to ecological/environmental (land use, extreme weather events, climate change), demographic (population growth and migration, conflict, behavior changes), evolutionary (microbial adaptation, vector resistance), public health (prevention programs, vector control, sanitation), and commercial (international trade) changes (Haines et al., 2006; Morse, 1995). Some of these changes in exposure are due to human actions while others are due to natural changes (Morse, 1995).

Many of the diseases thought to be most at risk of emergence or re-emergence due to climate change are vector-borne. Multi-host pathogens such as vector-borne diseases may be even more susceptible to climatic changes as the effects can be experienced by organisms in different stages of the parasitic life cycle. Most vectors, being arthropods, have an ectothermic physiology and are therefore more susceptible to the temperature fluctuations associated with climate change and therefore may be more likely to alter their ranges as previously inhospitable areas become habitable. As parasites move to new regions they may infect immunologically naïve populations, further exacerbating disease expansion (Parmesan and Martens, 2009; Patz and Olson, 2006).While predictions of such geographic expansions or shifts of disease and carriers due to climate change are common in the scientific literature (Epstein, 2001; Freed et al., 2005; Genchi et al., 2011; Gonzales et al., 2010; Mills et al., 2010; Nakazawa et al., 2007; Ostfeld, 2009; Parmesan, 2006; Parmesan and Martens, 2009; Polley and Thompson, 2009), no studies to date have directly linked current changes in climate to changes in the geography of vector-borne disease distributions. Prediction abounds, but confirmation remains elusive. This is no surprise since historical and current data on disease, vector, and reservoir host distributions is often nonexistent, scarce, or inaccessible.

Distributional data is difficult to collect even for large charismatic species, let alone small and repulsive organisms like pests and parasites. In addition, many species are not

well-recorded because they occur in remote regions, are cryptic, are not considered relevant for human health, or are considered noxious and thus do not benefit from amateur naturalists' recording schemes (i.e. citizen science). For meta-analyses, the best databases are from birds, butterflies, and plankton. There is a substantial lack of data on mammals, including rodents, and non-charismatic arthropods (e.g. phlebotomine flies, ticks, mosquitoes to the species level). Researcher access is also limited for disease data because of privacy concerns and HIPAA regulations.

Because leishmaniasis is an emerging and reemerging disease (Ashford, 2000), it makes sense to focus on this particular pathogen as a case study of disease expansion that is potentially driven by climate change. Of the emerging foci in the Mediterranean and Sudan, (Ashford, 2000) one particular recent focus has expanded quite far within the southern United States. During the past four decades reported cutaneous leishmaniasis has traveled northward and eastward, more than 560 km in the southern United States, primarily in Texas. Concurrently, Texas experienced hotter temperatures, increased precipitation, and greater yearly fluctuations in these variables (Nielsen-Gammon, 2008, 2011).

While potential causal drivers of the northeastward expansion were presented the first chapter of this dissertation as well as in the literature (Clarke et al., 2013), no study of these putative drivers has taken place. Here, we explored approaches to using species distribution models (SDMs) on data-poor records for the protozoan *Leishmania mexicana* in the southern USA. In this case, data scarcity is likely due to a lack of recording rather than actual rarity of the species and therefore recorded occurrence points likely greatly underestimate the actual distribution of the pathogen. No broad sampling study had been carried out within the geographic range of *Le. mexicana.*

The distribution of leishmaniasis in the United States clearly shows a northeastern trend (Figures 2.1 and 2.2). However, experts disagree as to whether the pathogen is truly spreading. Wright et al. describes it as colonizing a new region (Wright et al., 2008), whereas McHugh argues it is most likely endemic and only now being reported due to increased surveillance and sylvatic habitat encroachment by humans (McHugh, 2010).

Multiple drivers have been hypothesized, but none have been definitively linked to the observed expansion. Here we investigate the degree to which recent changes in temperature and precipitation can explain the geographical expansion of cutaneous leishmaniasis in the United States.

We used occurrence data from two time periods, 1982-1994, and 2001-2015, to construct species distribution models of *Le. mexicana* with climate variables derived from 1982-1994 and 2001-2014. Forward and backward projections were created to estimate the transferability of the resulting models, as well as the change in distribution due to climate.

METHODS

Species distribution modeling was chosen as an exploratory means to discover if suitable habitat for *Leishmania mexicana* had expanded in the southern United States during the recent past, the period ranging from 1982 to 2014. I used Maxent 3.4.0 to model the distribution of the pathogen (Phillips et al., 2017a). Maxent is a statistical software package that builds a correlational model between species occurrences and environmental variables, attempting to minimize the relative entropy between species presence probability density and landscape density distributions in covariate space (Elith et al., 2011). The output is a map or grid of cells where each cell is the predicted probability of presence for a species within geographic space that has been projected from environmental covariate space. Functions are also calculated that show species relationships with individual environmental variables. I chose Maxent because it has outperformed other species distribution modeling methods with presence-only data (Barve et al., 2011; Elith et al., 2006; Graham et al., 2008; Phillips and Dudík, 2008).

The two temporal periods chosen encompass the two main peaks in outbreak of the disease in human and pet populations. The first period ranged from 1982-1994 and the second period ranged from 2001-2014. The extent chosen represents the south-central United States consisting of states where leishmaniasis has been primarily observed, bordering states, and states where it could be expected to spread.

However, since much of the occurrence data encompassed humans with a greater ability to travel or disperse over long distances than is likely for the woodrat reservoirs or sand fly vectors it made more sense to use a coarse resolution model. Dispersal of one species of woodrat reservoir ranges from 5-210 meters (Merkelz and Kerr, 2002; Raun, 1966) while sand fly species from other areas of the world may disperse up to 960 meters (Casanova et al., 2005). Thus, it is possible humans who contracted the disease were exposed at a site quite distant from their home address.

The first model used data from 1982-1994 to construct the model, and projected occurrences from 1982-1994 to 2001-2014. This model used 26 cases/isolations of the pathogen documented in the literature in humans and cats (Barnes et al., 1993; Craig et al., 1986; McHugh, 2010). However, there were multiple problems with this data. It was unclear where the parasite was acquired in 7 of the cases as there was travel to multiple regions of Texas and/or Mexico. After removing these "suspect" cases, duplicate points and redundant points falling within the same cell were also removed resulting in a total of 11 localities used for model construction.

For the second model, data from 2001-2014 was used to build the model, then occurrences were projected backward from 2001-2014 to 1982-1994. For this we used 24 geographic coordinates from humans, cats, and dogs obtained from the Texas Department of State Health Services (Vilcins, 2016) and the literature (Kipp, 2015; Trainor et al., 2010). DSHS case data was categorized as locally acquired, travel associated, or unknown. Only cases categorized as locally acquired were used in this analysis. Duplicate geographic records were removed and points that had fallen off the grid due to aggregation of cells reducing raster border area were moved to the nearest cells.

Environmental variables including precipitation, mean temperature, minimum temperature, maximum temperature per month were obtained from PRISM at a 4 km resolution (Oregon State University) for our periods of interest (1982-1994 and 2001-2014). To create the 19 bioclimatic variables (Table 2.1), the Biovars function in the Dismo package in R was used. Data were resampled using cubic convolution for the 19 quantitative variables in ArcMap 10.

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

Table 2.1: Table of derived bioclimatic variables used in construction of species distribution models.

Maxent was run using the cloglog function, random seed, and a training/test percentage of 75:25 with bootstrap resampling. Both models were replicated 100 times and the average of those models analyzed in the following results and discussion. The new cloglog function in Maxent 3.4.0 allows the output to be interpreted as probability of presence (Phillips et al., 2017a, 2017b).

The centroid of the distribution for each model weighted by probability of presence was calculated in both time periods for the forecasted model and the hindcasted model. The following formula was used where *i* represents the cell, *x* represents the longitudinal coordinate, *y* represents the latitudinal coordinate, and *p* represents the probability of suitable habitat, to calculate the centroids of the distributions:

$$\frac{\sum_i x_i\, p_i}{\sum_i p_i} \qquad \frac{\sum_i y_i\, p_i}{\sum_i p_i}$$

RESULTS

1982-1994 model projected forward to 2001-2014

The model built upon data from 1982-1994 and then projected to 2001-2014 showed high probability of presence in the range of the past known occurrences of leishmaniasis (Figure 2.1) and showed a slight northward shift of 21.7 km from the past (30.87926111°, -98.52513969°) to 2001-2014 (31.075013°, -98.533212°) (Figure 2.2). The difference in probability or habitat suitability between the two time periods was calculated. The difference map (Figure 2.3) shows a decrease in suitable habitat in the south and a more heterogeneous general increase in suitability in the north. A shift of ~21 km northward was calculated via the weighted centroid analysis.

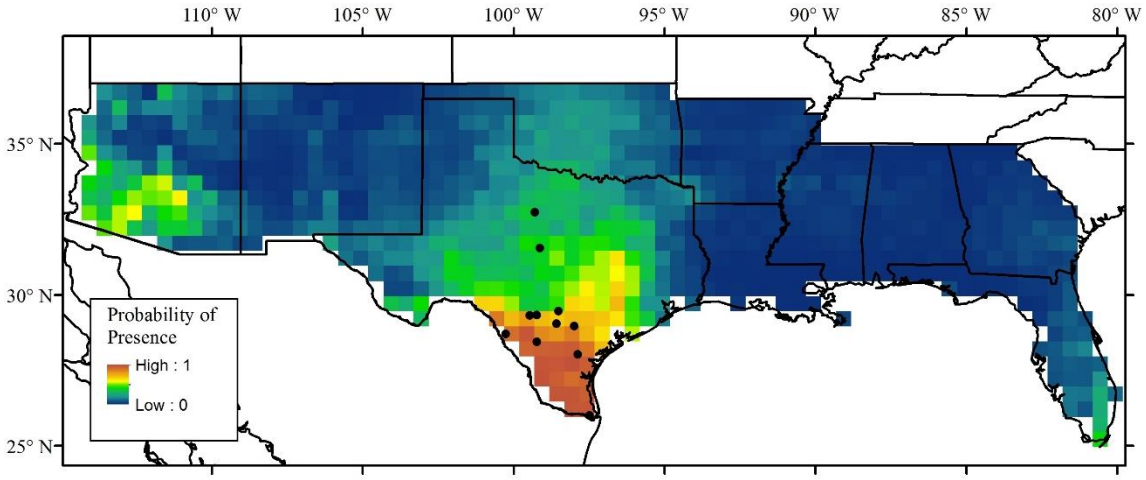

Figure 2.1: Species distribution model from 1982-1994. Black points represent cases used to construct the model from the same time period.

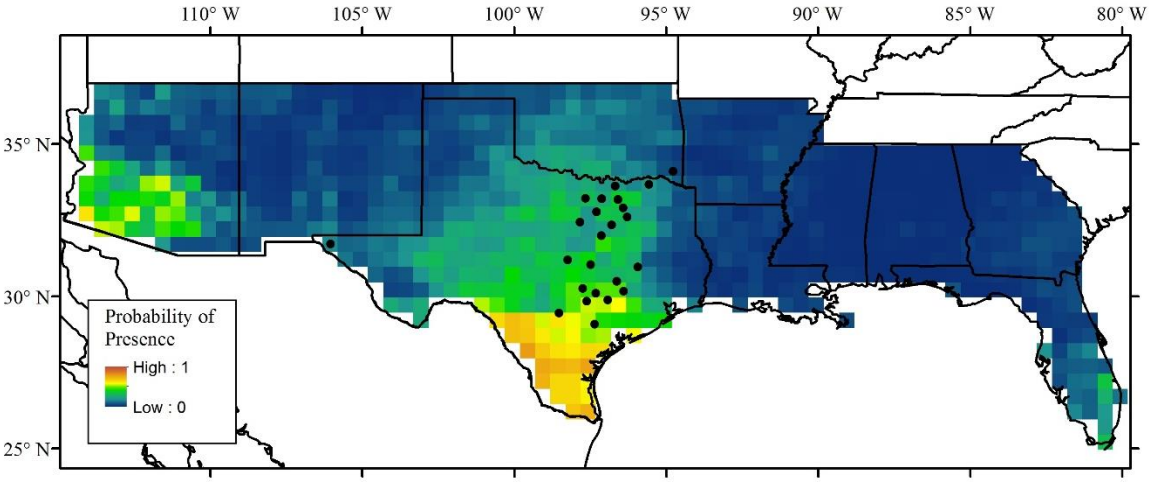

Figure 2.2: The model from 1982-94 projected to climatic variables from 2001-2014. Black points represent human and cat cases from 2001-2015.

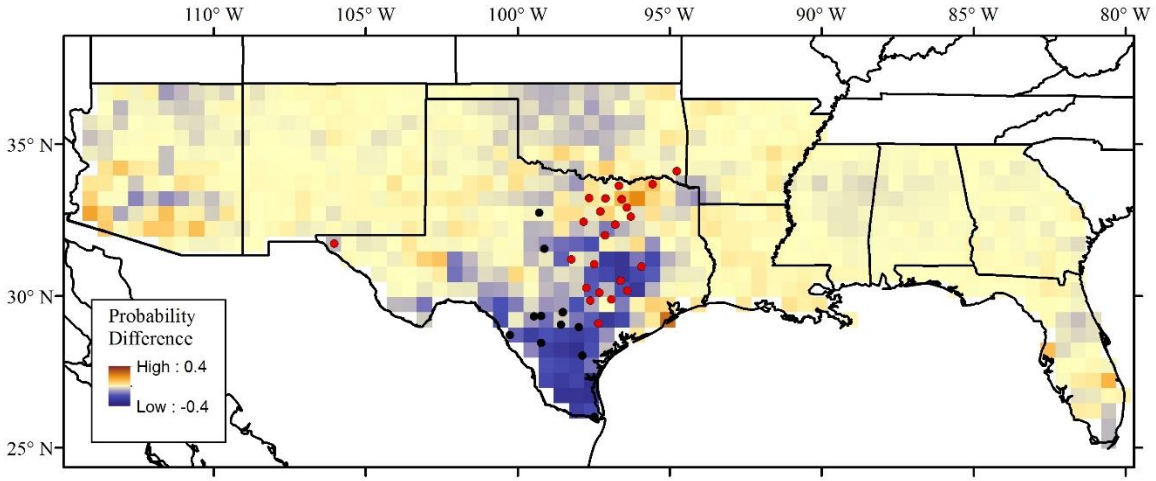

Figure 2.3: This map represents the difference in probability of presence between the past and the present/future, using the model built upon past data (*i.e.* the differences between Figure 2.1 and Figure 2.2). Black points represent leishmaniasis cases or *Le. mexicana* isolations from 1982-1994 and red points represent those from 2001-2015. Dark blue areas underwent a decrease in probability while orange areas experienced a gain in probability of presence.

The model built from 1982-1994 had an AUC of 0.953 based on occurrence data used to build the model. The model was then projected to 2001-2014 using data from that same time period and found to have an AUC of 0.854. The AUC (area under the receiver operating characteristic curve) is a metric used to examine how well a model performs based on its ability to correctly predict occurrences and absences. Values above 0.5 mean the model is performing better than random and values below 0.5 means a model is performing worse than random. Temperature variables were found to be most important to this model. Annual mean temperature was the top percentage contributing variable (46.7%) and one of three top variables in permutation importance (24.1%). As temperature increased, probability of presence increased. Precipitation of the driest month was the second top percentage contributing variable (22.9%) and highest variable in permutation importance (24.8%) and as it increased, probability of presence decreased. Precipitation of the warmest quarter had the third highest percentage contribution (12.5%) and the fourth highest contribution of permutation importance (9.1%). Lower amounts of precipitation predicted higher probability of presence. Temperature seasonality was the third most

important variable in permutation importance (11.4%). Interestingly, up to a certain threshold, increases in seasonality or monthly temperature variation did not seem to affect probability of presence; however, after a midpoint was reached, presence dramatically declined.

2001-2014 model projected backwards to 1982-1994

The model built from 2001-2014 data projected backwards to the climate of 1982-1994 showed high habitat suitability/probability of presence in the range of the recent known occurrences of leishmaniasis (Figure 2.4) and captured the range of new cases. The past model projected from 2001-2014 also captured the southern range of the disease but overestimated the past range (Figure 2.5). There was a northeastward shift of the weighted distributional centroid from the past (30.939858°, -97.547133°) to 2001-2014 (31.657715°, -96.869388°) of 103 km. The difference map shows the change in probability between the two time periods (Figure 2.6).

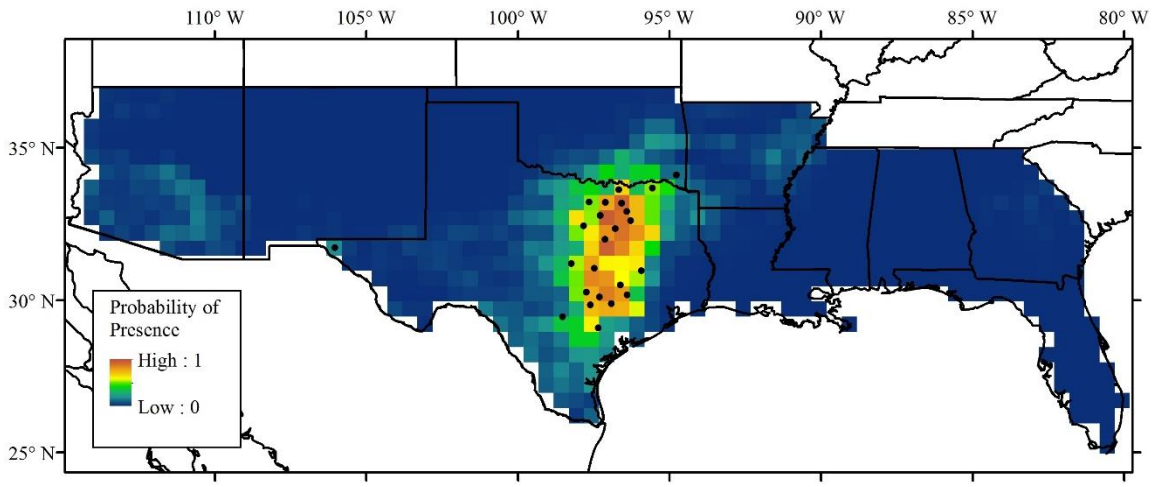

Figure 2.4: The species distribution model based on 2001-2014 climatic variables. Black points represent cases used to construct the model from 2001-2015.

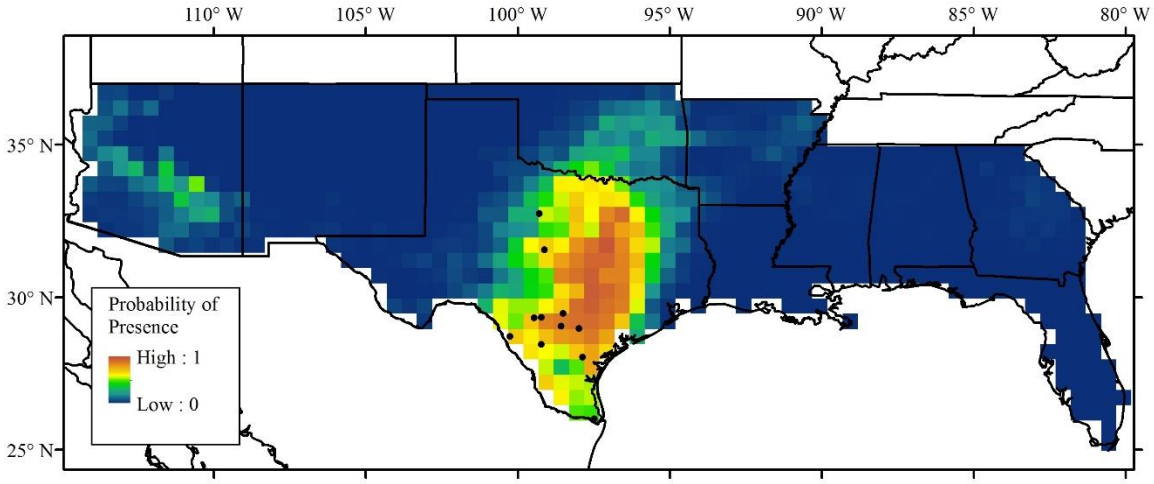

Figure 2.5: The model from 2001-2014 projected to climatic variables from 1982-1994. Black points represent human and cat cases from 1982-1994.

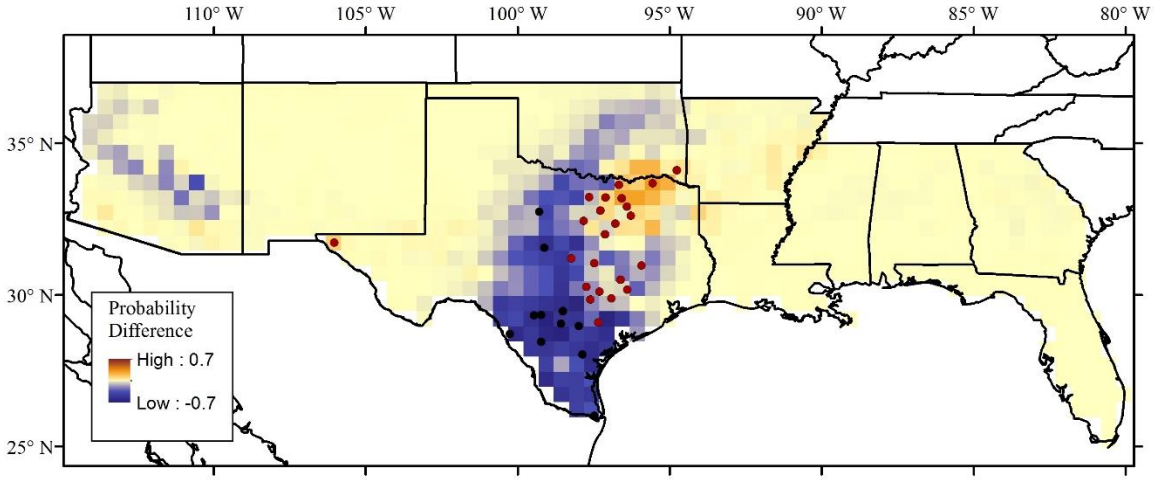

Figure 2.6: This map represents the difference in probability of presence between the past and the present/future. Black points represent leishmaniasis cases or *Le. mexicana* isolations from 1982-1994 and red points represent those from 2001-2015. Dark blue areas underwent a decrease in probability while orange areas experienced a gain in probability of presence.

The model built from 2001-2014 had an AUC of 0.943 based on occurrence data used to build the model. The model was then projected to 1982-1994 using data from that same time period and found to have an AUC of 0.977. Variables found to be most important to the second round of models were precipitation associated variables. The variables with

the highest percentage contribution and permutation importance differed substantially from the variables in the earlier analysis. Most of these variables showed parabolic curves associated with increased probability of presence as opposed to linear increases or decreases to a threshold like temperature associated variables. Annual precipitation represented the top contributing percentage variable (36.2%) and contributed very little to permutation importance; however, varying this variable caused little change to probability of presence. Precipitation of the coldest quarter contributed 18.5% and 19.5% to permutation importance. Probability of presence peaked at ~170 mm. Precipitation of the driest month contributed the next highest amounts 16.9% and 15.3% to permutation importance. Probability of presence peaked at ~10 mm which represents the lower range of precipitation values found in the model extent. The mean temperature of the driest quarter contributed 11.5% but very little to permutation importance and peaked at 17°C. Precipitation of the warmest quarter contributed 29.3% to permutation importance and probability of presence peaked at 215 mm.

Comparison of climate states between two periods

A principal components analysis of climate variation between the two different time periods of peak cases (1982-1994, 2001-2015) was carried out using the 'stats' package in R (R Core Team, 2013). Principal components were calculated from the 19 climatic variables of both time periods. After calculating components, case localities were used to obtain specific climate variables for each case from its respective time period and projected into principal component space (Figure 2.7). The first period of cases overlaps with the second period in climate space; however, there are also distinct areas of disjuncture.

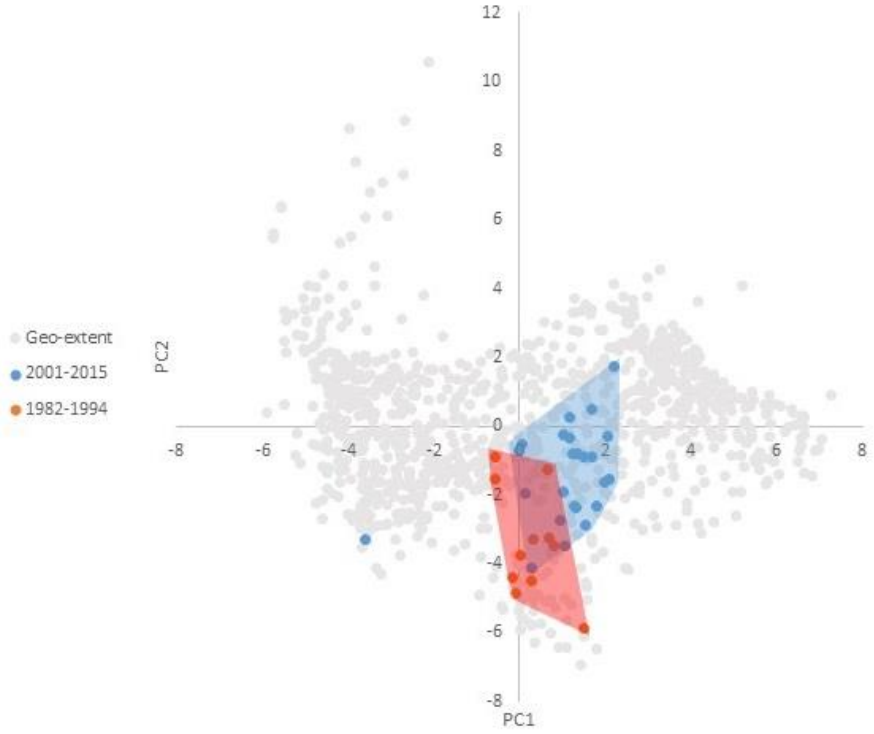

Figure 2.7: Gray points represent the overall distribution of the climate space used to construct the principal components. Orange points represent the cases from 1982-1994 while blue points represent cases from 2001-2015. The blue point not included in the climatic space of 2001-2015 represents the El Paso locality of *Le. mexicana.*

The highest loadings associated with the first two principal components were measured. The highest loading variables observed for the 1st principal component corresponded to mainly precipitation derived variables (two variables were scored the same: precipitation of driest quarter, 0.320, and for annual precipitation, 0.320). The 2nd principal component consisted mainly of temperature derived variables (the top scoring variable was mean temperature of the warmest quarter, -0.452). Interestingly enough, annual precipitation was found to be a top contributing variable in the hindcasting models and 1st principal component.

DISCUSSION

Both forecasting and hindcasting models capture the northern spread of the pathogen and the cases documented in the El Paso area but to different degrees. The 1982-

1994 occurrence data was sparse and limited to 11 unique localities after stringent data processing procedures. However, these cases were documented during a time when leishmaniasis was not a reportable disease in Texas. This may have created a spatially biased data set of poorer quality than more recent data. Maxent is said to be less sensitive to this problem, with claims that as few as 5 data points (occurrence records) are able to build a successful model (Pearson et al., 2007). Cases from 2001-2015 represent a much more comprehensive set of occurrence data as reporting at a statewide level was possible, so those models are likely more reliable. There were also more points available for input into models.

We hindcasted the expansion from an historic starting point using a novel dataset on northward expansion of cutaneous leishmaniasis in Texas. The hindcasted model captures disease distribution well in both time periods; although it also predicts habitat in central Texas well beyond the documented range in 1982-1994 (Fig 2.5). It is possible that the pathogen was present in these areas but undocumented. Alternatively, the parasite may have lagged when spreading to these areas of suitable habitat. It also predicts a range shift of the pathogen and a contraction of suitable habitat from 2001-2014 (Fig 2.4). It is necessary to note that future models based on various climate models of the vectors and reservoirs suggest an expansion dramatically north and east of Texas (Gonzales et al., 2010). This model also scored a higher AUC metric than the forecasted model. Annual precipitation was one of the highest loading scores in the PCA and the highest contributing variable to the Maxent model. Precipitation values clustering around 900 mm predicted the highest probability of presence. Annual precipitation in Texas has generally been increasing since the 1980s in the eastern 2/3 of the state with average precipitation rising from 850 mm to 870 mm. It is important to note that soil is becoming drier (Environmental Protection Agency, 2016). Future studies should include soil variables

Overall, both models captured the recent range of the parasite, albeit, to different degrees. In order to discern whether the southern niche of the species moved northeastward, a niche shift via species jump took place, or sampling bias compromised the models, a principal components analysis of climate space was carried out. The principal components

analysis suggests that climate change has contributed as evidenced by the overlapping area and that a species jump may have also occurred as evidenced by the non-overlapping areas in their expected locations. The disease may have shifted into a new vector, reservoir, or both; however, analyzing this hypothesis was not a goal of this research project. However, the sampling bias explanation for the range shift cannot be definitively refuted.

The question which remains is whether the extent to which the ranges don't overlap reflect changes in ecology or changes in recording? It is unclear whether an overall range shift has occurred because cutaneous leishmaniasis cases are recorded in northern Mexico in states that border Texas: Coahuila de Zaragoza, Nuevo Leon, and Tamaulipas (Pérez-Vega et al., 2009). However, the hindcasted model does suggest that the range has shifted or new foci have developed as southern Texas has experienced a dramatic decrease in probability of presence in both models (Figures 2.3 and 2.7). Further models of vectors and reservoirs are warranted to understand the dynamics of this emerging disease.

Chapter 3: Phlebotomine fly diversity and distributions in Texas

ABSTRACT

Phlebotomine flies serve as the primary vector of leishmaniasis. Thirteen species are found in the United States and Texas, in particular, has a very high phlebotomine fly diversity with 8 species reported before this study. Only south Texas had been sampled in the past thus leaving several large geographical gaps, especially in areas of recent cutaneous leishmaniasis emergence. Additionally, morphological identification of phlebotomines yielded inconsistent results so a new barcoding method using *cyt b* was created to identify difficult specimens with ambiguous identifications. Overall, the barcoding analysis suggests major revisions to the taxonomy of phlebotomine flies in the United States and 2 potential new species have been identified. The analysis also documented multiple specimens collected beyond the previously known range limits of species. Importantly, some of these range expansions occurred in the same regions of increased *Leishmania mexicana* activity.

INTRODUCTION

The importance of the study of vector-borne disease in regards to climate change and emergence has already been discussed in chapters 1 and 2. There are other reasons why the study of vector-borne diseases should be carried out. First, there are obvious human health implications: more than half of the world population is at risk of contracting a vector-borne disease (World Health Organization, 2004). Secondly, vector-borne diseases represent a unique and complicated set of biotic and ecological interactions. Vector-borne diseases connect different organisms that otherwise may have no contact with one another and make the life histories of one organism dependent on another. There are obvious co-evolutionary implications for multiple species within an ecosystem that has vector-borne pathogens, especially for the pathogen itself and its transmission and virulence. The vectors, most commonly arthropods, within these multifarious webs of interconnectivity play a key edge building role in connecting different organisms.

The most commonly known vectors are mosquitoes, fleas, and ticks that have widespread distributions. Less commonly known vectors are triatomine bugs and phlebotomine flies. Phlebotomine flies, formerly colloquially called sand flies, are the vector of the leishmaniases. They are also important disease vectors of a variety of other pathogens: phleboviruses such as Rio Grande virus and Massilia virus; rhabdoviruses such as vesicular stomatitis virus; and bacteria such as *Bartonella*. Phlebotomine flies are hematophagous dipterans of the suborder Nematocera distributed across the tropical, subtropical, and temperate regions of Asia, Africa, Europe, North America, South America, and Australia (Killick-Kendrick, 1999). Of the 900 described species and sub-species of phlebotomine flies (Depaquit, 2014), only 50-70 are described as potential vectors of leishmaniasis (Kamhawi, 2006; Ready, 2013). In order to understand the disease ecology of leishmaniasis, primary research on vectors must be made a priority.

Recent habitat suitability models suggest that the vectors capable of transmitting leishmaniasis within the United States will expand their ranges substantially throughout the 21st century (Gonzales et al., 2010). Very little phlebotomine sampling has taken place in the southern United States in areas that experienced recent geographic expansion of leishmaniasis such as Texas and Oklahoma. While sampling of phlebotomine flies increased in the 1980s and 1990s due to a spate of cases in the Edwards Plateau, recent sampling has been lacking.

Through extensive new field sampling, this study aims to increase knowledge of the ranges of sand fly species in the Southern United States, in particular, Texas, the seat of increased leishmaniasis activity. We used both morphological and molecular techniques to identify known species, look for new species and construct a new phylogenetic tree. We then compiled a list of phlebotomine flies found in the United States (Table 3.1) and likely leishmaniasis vectors (Table 3.2) using updated information from the literature and from our own field studies. Field studies are particularly important because the literature focused mainly on sites within south Texas and no new collections had taken place in the areas of cutaneous leishmaniasis emergence.

Sand fly species	*Location*	*WHO Vector Criteria*	*Suspected Host Preference*	*References*
D. anthophora	Mexico to USA, AZ, TX	2, 3, 4, 5	woodrat (*N. micropus*)	(McHugh et al., 1993; Mead and Cupp, 1995; Perkins et al., 1987; Young and Perkins, 1984)
D. aquilonia	USA to Canada, CO, TX, WA		marmot	(Claborn et al., 2009; Young and Perkins, 1984)
Lu. cruciata	Panama to SE Mexico, FL, GA	1, 4, 5	human	(Williams, 1966; Young and Perkins, 1984)
Lu. diabolica	SW Mexico to TX	1, 4, 5	human	(Lawyer and Young, 1987; Lawyer et al., 1987; Young and Perkins, 1984)
Lu. tanyopsis	AZ			(Young and Perkins, 1984)
M. apache	AZ, CO, NM, WY			(Alsuhaibani, 1990; Herrero et al., 2004; Reeves et al., 2008; Schmidtmann et al., 2002; Young and Perkins, 1984)
M. californica	AZ, CA, TX, WA		lizard, snake	(Young and Perkins, 1984)
M. oppidana	Mexico to Canada, CO, MT, TX, WA			(Eads, 1978; Young and Perkins, 1984)
M. stewarti	Mexico to USA, CA			(Young and Perkins, 1984)
M. vexator	Mexico to Canada, AL, AR, CA, CO, CT, FL, GA, KS, KY, LA, MD, MS, MO, MT, NM, OH, OK, TN, TX, VA, WA, WY		lizard, snake	(Claborn et al., 2009; Minter et al., 2009; Weng et al., 2012; Young and Perkins, 1984)
Pi. xerophila	USA, CA			(Young and Perkins, 1984)
Ps. shannoni	Argentina to USA, AL, AR, DE, FL, GA, KS, KY, LA, MD, MS, MO, NC, OH, SC, TN, TX	1, 3, 4, 5	human, sloth, some birds	(Christensen and de Vasquez, 1982; Claborn et al., 2009; Lawyer and Young, 1987; Lawyer et al., 1987; Minter et al., 2009; Pech-May et al., 2010; Weng et al., 2012; Young and Perkins, 1984)
Ps. texana	Honduras to USA, TX		armadillo	(Christensen and de Vasquez, 1982; Young and Perkins, 1984)

Table 3.1: Phlebotomine species, distribution, WHO vector criteria, and feeding preferences

SAND FLY SPECIES	*LOCATION*	*WHO VECTOR CRITERIA*	*SUSPECTED HOST PREFERENCE*	*REFERENCES*
D. anthophora	Mexico to USA, AZ, TX	2, 3, 4, 5	woodrat (*N. micropus*)	(McHugh et al., 1993; Mead and Cupp, 1995; Perkins et al., 1987a; Young and Perkins, 1984)
Lu. cruciata	Panama to SE Mexico, FL, GA	1, 4, 5	human	(Williams, 1966; Young and Perkins, 1984)
Lu. diabolica	SW Mexico to TX	1, 4, 5	human	(Lawyer and Young, 1987; Lawyer et al., 1987; Young and Perkins, 1984)
Ps. shannoni	Argentina to USA, AL, AR, DE, FL, GA, KS, KY, LA, MD, MS, MO, NC, OH, SC, TN, TX	1, 3, 4, 5	human, sloth, some birds	(Christensen and de Vasquez, 1982; Claborn et al., 2009; Lawyer and Young, 1987; Lawyer et al., 1987; Minter et al., 2009; Pech-May et al., 2010; Weng et al., 2012; Young and Perkins, 1984)

Table 3.2: An updated table of phlebotomine flies found in the United States that fit several WHO vector criteria.

METHODS

69 field sites (including EcoLab sites, private properties, wildlife management areas, and state parks) across the state of Texas were sampled for phlebotomine flies and subsequently, infection with *Leishmania mexicana.* Sites were selected based on accessibility, regional proximity to new disease cases, and whether or not regional sampling for phlebotomine flies and *Leishmania* had taken place. At each site, 1-20 CDC mini-light traps were set for 1 or 2 nights on different dates. Some sites were visited more than once over a period of 2 years. GPS coordinates were recorded for each trap.

Phlebotomine flies were separated from other insects caught in the traps and stored in 95% ethanol in a -20°C freezer until DNA extraction and morphological identification took place. Phlebotomine flies were identified morphologically using the keys of Young and Perkins (Young and Perkins, 1984) and Galati or molecularly using the cytochrome b (*cyt b*) gene. New World sand flies are currently undergoing taxonomic revision. Recently, Galati has suggested splitting the New World genus *Lutzomyia* into several genera. Galati's taxonomic classification will be used in the rest of this document.

Sand flies that could not be identified morphologically or had ambiguous preliminary identifications were identified molecularly using fragments from the *cyt b* gene according to standard barcoding procedure. Barcoding of species is a technique first introduced by Hebert in 2003 (Hebert et al., 2003). Barcoding represents a way to identify species using universal genetic markers. The most commonly used genetic mark is COI but other genes have also been proposed such as internal transcribed spacers 1 and 2, 16sRNA, and cyt b. Within sand fly studies of systematics, the *cyt b* gene is the most widely used marker (Depaquit, 2014). The key to genetic barcoding is the occurrence of high levels of interspecific variation and low levels of intraspecific variation. Barcoding not only acts as a means of species identification but also can inform future phylogenetic analyses, although there is often not enough information present in the short sequences of commonly used mitochondrial DNA to assess deep seated phylogenetic splits.

Sand flies were stored in 95% EtOH within -20°C freezers until specimen preparation for mounting and DNA extraction. The first 100 specimens were prepared for mounting via dissection of the head and genitalia. The thorax and first 4 segments of the abdomen were crushed. DNA was extracted according to the Qiagen DNeasy Blood and Tissue DNA extraction kit protocol. The remaining specimens underwent whole sand fly DNA extraction that kept the specimens intact via submersion of the sand fly in proteinase k and ATL solution. At first 200 µl elutions were used in the final step according to kit instructions but this resulted in very low DNA concentrations. The protocol was subsequently modified to 100 µl and then finally 75 µl for final elutions.

Primers N1N-PDR and C3B-PDR were used to amplify a 500 bp region of the cyt b gene (Esseghir et al., 1997). PCR was carried out using a touch down technique with an initial temperature of 94°C for 3 minutes and finishing with a final extension of 68°C for 10 minutes. The following PCR temperature profile was used: 5 cycles with 30 sec 94 °C, 30 sec 40 °C, 1 min 68 °C and 35 cycles with 30 sec 94 °C, 30 sec 44 °C, 1 min 68 °C. A 1% agarose gel containing ethidium bromide was used to visualize amplicons afterward.

Sequencing was carried out by the University of Texas at Austin DNA sequencing facility. Sequences were cleaned and aligned using Geneious 6.1.8 software and SeaView

(Gouy et al., 2010). A gap of 4-6 nucleotides was found in the alignment at the 322nd base pair position. After this gap there was a lack of sequence diversity so sequences after the gap were removed.

A phylogenetic tree was constructed using the MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) plugin developed by Marc Suchard with the following default parameters: Generalised Time Reversible (GTR) substitution model; and Gamma rate variation with 4 categories. The Markov Chain Monte Carlo default settings were as follows: 1,100,000 chain length with 4 heated chains heated to a temperature of 0.2; a subsampling frequency of 200; a burn-in length of 100,000; a random seed of 11,785. The only difference made from default settings was the selection of unconstrained branch lengths.

RESULTS

Species identification and phylogeny

Phlebotomine flies were collected at 41 of the 69 sites sampled from 2013 through 2015 (Figure 3.1). 814 trap nights took place with catches ranging from 0 to 197 flies. Phlebotomine flies were collected belonging to 8 morphologically identified species, however molecular analysis indicated our samples belonged to only 6 species or lineages (Figure 3.2). The species identified morphologically were *Dampfomyia anthophora, Lu. diabolica, Micropygomyia apache, M. californica, M. oppidana, M. vexator, Psathromyia shannoni*, and *Ps. texana*.

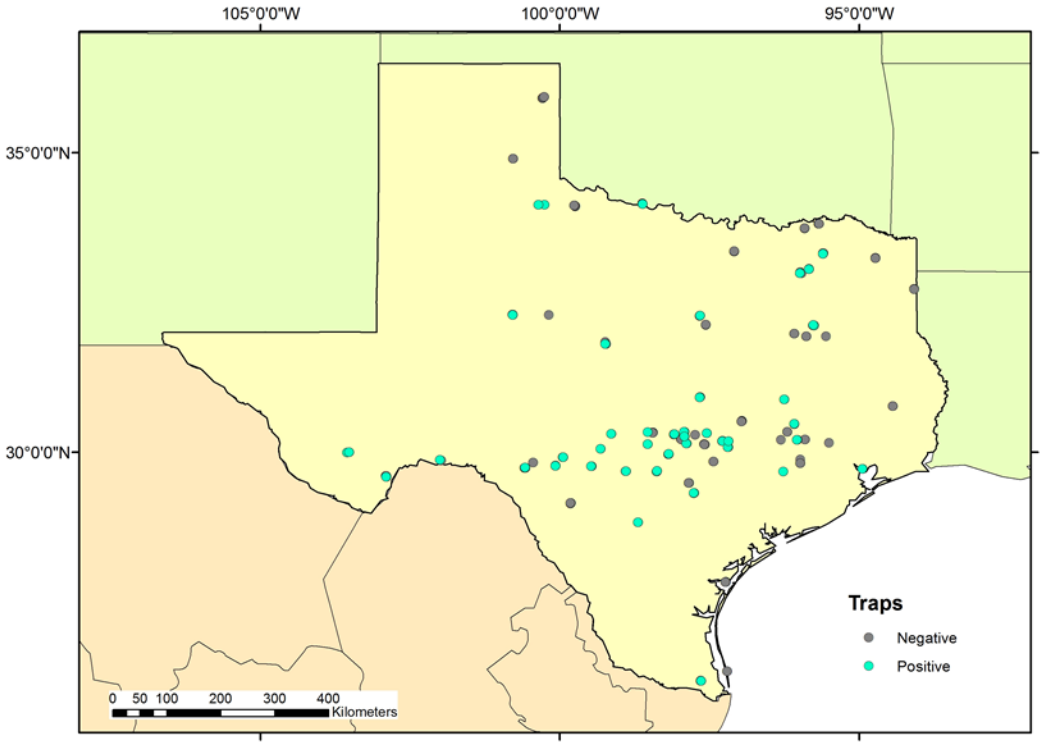

Figure 3.1: Turquoise points represent locations where phlebotomines were captured while gray points represent locations where phlebotomines were not captured.

A bar-coding analysis determined there to be 6 lineages although experts identified eight species morphologically (Figure 3.2). One clade, which will be referred to as the *M. spp.* complex, contains five morphologically identified species and two specimens that could not be morphologically identified. One of the specimens that appears morphologically to be *Ps. texana* actually falls in the complex. This may have been due to inadvertent contamination or switching/mislabeling of samples during processing.

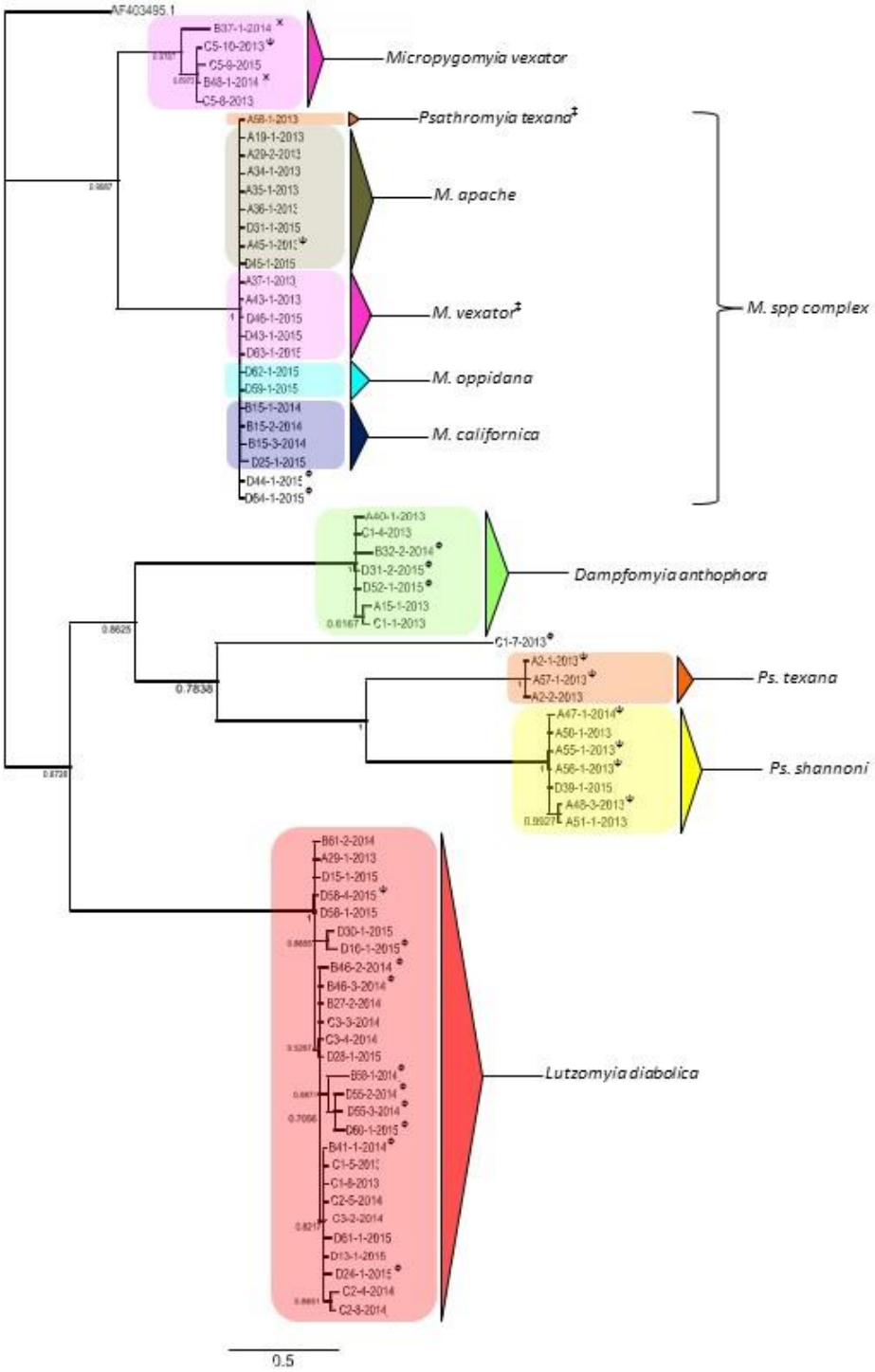

Figure 3.2: Cytochrome b Bayesian Tree of various phlebotomine specimens. Different colors represent different morphological species identifications. The asterisk (*) denotes

specimens identified as *Micropygomyia vexator occidentis,* a subspecies of *M. vexator*. The double dagger symbol (‡) denotes specimens identified morphologically as one species but that come out in a different clade in the molecular analysis; for example, specimen A58-1-2013, was identified morphologically as *Ps. texana* but falls into the *M. californica/vexator* clade. The small phi symbol (ϕ) represents specimens that were unable to be identified morphologically due to missing genitalia or heads but were able to be identified via molecular analysis. The small Greek Phi symbol (φ) represents specimens clearly identified by experts. Specimen C1-7-2013 appears singularly on its own branch, this specimen was also found to have an Acari mite parasite but Blasting the specimen showed the DNA to be most similar to various *Lutzomyia* specimens. AF403495.1, a *Lutzomyia longipalpis* specimen was used as the outgroup.

Species occurrence data

After morphological and molecular identification, maps of phlebotomine fly distributions were created. *D. anthophora* meets 4 out of 5 of the WHO's criteria for vector identification. *D. anthophora* is a small and pale sand fly ranging in length from 0.9 to 1.3 mm. It has previously been recorded in southern, central and western Texas (Figure 3.3) but in this study was also found in the Panhandle region of Texas and further east. The Panhandle occurrence greatly expands the northern range limit of this species.

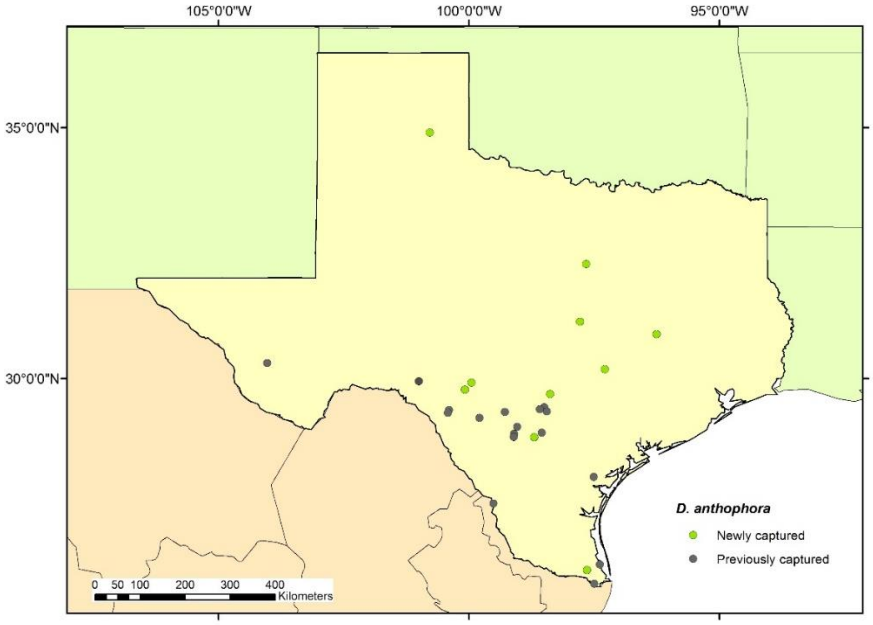

Figure 3.3: Green points represent locations where *Dampfomyia anthophora* were newly collected for this study. Gray points represent past occurrence data from Young and Perkins and Moo-Llanes (Moo-Llanes et al., 2013; Young and Perkins, 1984).

Lutzomyia diabolica is one of the potential vectors of leishmaniasis in Texas meeting 3 of 5 WHO criteria. It tends to be a large, dark, and hairy fly ranging in size from 1.3 to 2.1 mm in length. Before this study, it had been found mainly in southern and central regions of Texas with a few occurrences in western Texas, the Panhandle, and the Gulf Coast (Figure 3.4). This study documented new occurrences in in northern and northeastern Texas in the same regions where leishmaniasis is currently being documented.

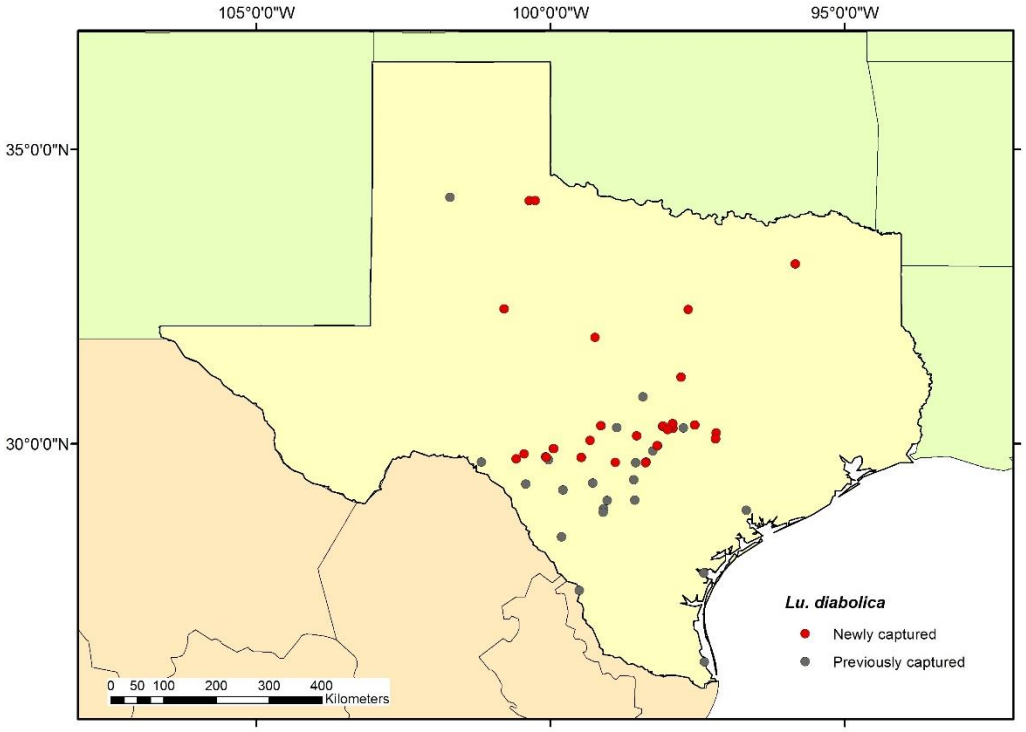

Figure 3.4: Red points represent locations where *Lutzomyia diabolica* were newly collected for this study. Gray points represent past occurrence data from Young and Perkins and Moo-Llanes (Moo-Llanes et al., 2013; Young and Perkins, 1984).

Micropygomyia apache tends to be a light colored and very hairy fly ranging from 1.3 to 1.6 mm in length. *M. apache* had previously only been recorded in Arizona, Colorado, New Mexico, and Wyoming (Alsuhaibani, 1990; Herrero et al., 2004; Reeves et al., 2008; Schmidtmann et al., 2002; Young and Perkins, 1984) making this its first recording in Texas (Figure 3.5). It was reported from 3 different counties in Texas: Bell, Blanco, and Somervell. Bell and Blanco counties are in central Texas while Somervell is

just southwest of the Dallas-Ft. Worth metroplex. These occurrences greatly expand the known range of this species into a new state.

According to our phylogenetic analyses, this species occurs in a clade in a polytomy with 3 other *Micropygomyia* species: *M. californica, M. oppidana,* and *M. vexator.* Interestingly, *M. apache, M. oppidana, M. stewarti,* and *M. vexator* are thought to occur as a species complex.

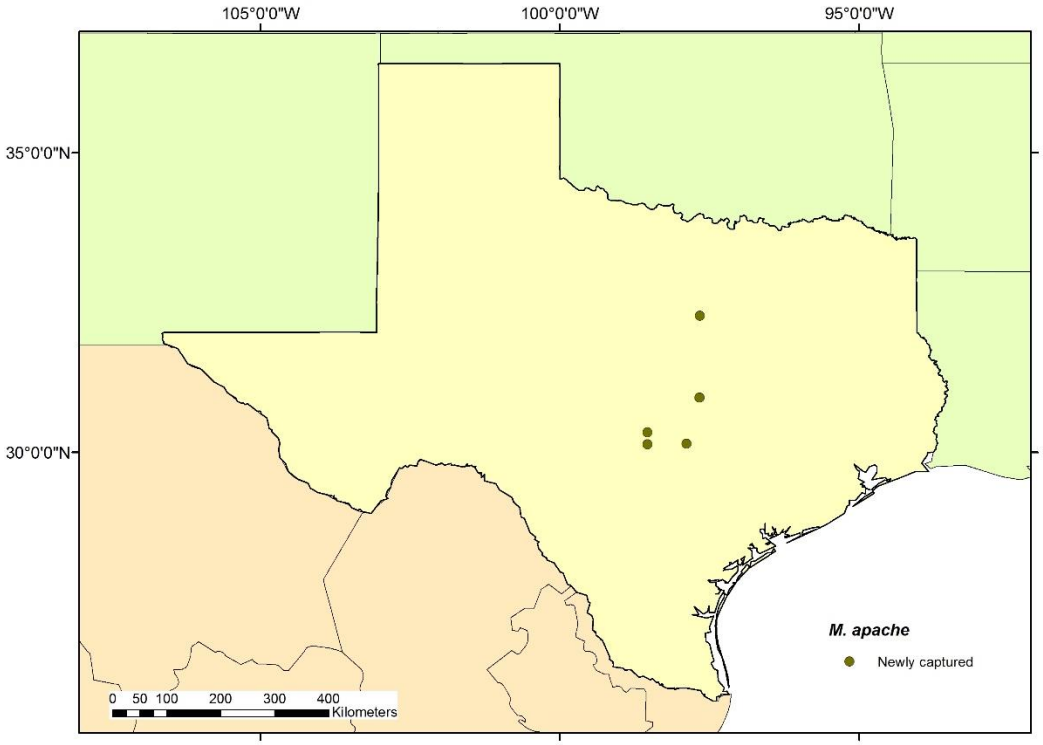

Figure 3.5: *Micropygomyia apache* captures are denoted by the olive color and represent a newly documented species in Texas with no previous captures in Texas.

M. californica is a pale fly approximately 1.4 mm in length found mostly in western portions of the United States. There is some uncertainty surrounding its taxonomy as well based upon morphological features in the Texas collections (Figure 3.6). Another southern species, *M. chiapanensis*, occurring in Central America is nearly indistinguishable from *M. californica.* These two species may actually comprise one species. Unfortunately, no sequence entries of either species are available in GenBank for phylogenetic analysis. This

species also clustered within the *M. vexator/apache/oppidana* clade based on *cyt b*. This result is surprising given it does not fall into the *vexator* species group of *Micropygomyia*.

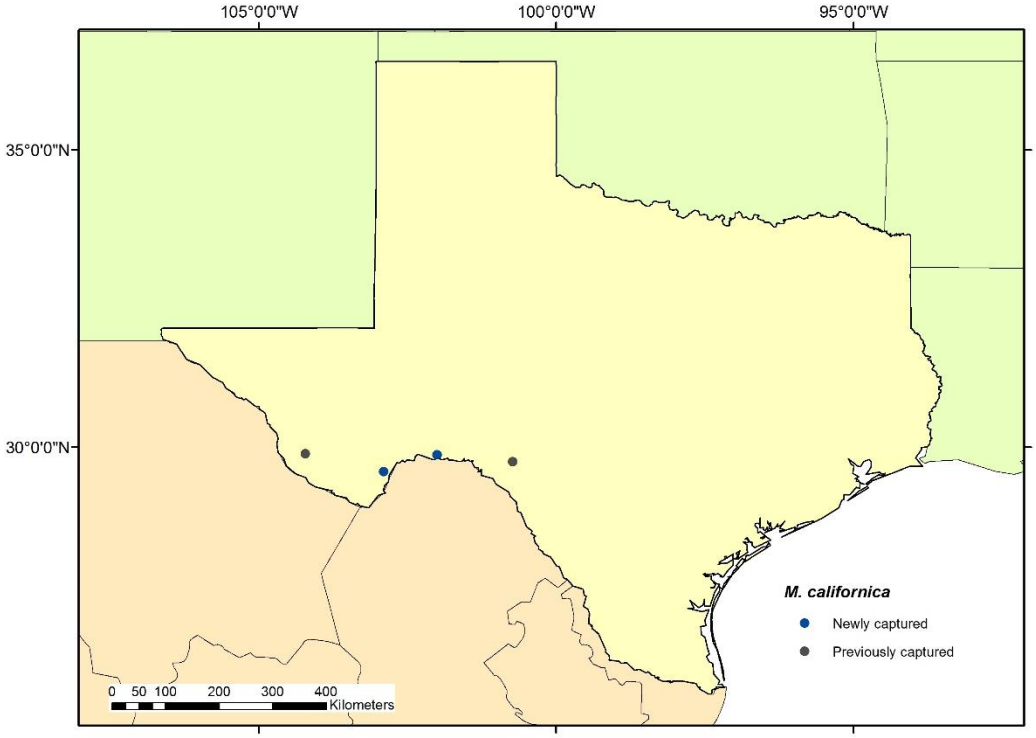

Figure 3.6: *Micropygomyia californica* is represented by navy blue occurrence points while previously captures flies are represented by gray occurrence points from Young and Perkins (Young and Perkins, 1984).

M. oppidana is a pale fly measuring 1.5 mm in length. *M. oppidana* is a widely distributed western species ranging from Mexico to southern Canada and has previously been documented in Colorado, Montana, Texas, and Washington (Eads, 1978; Young and Perkins, 1984). In this study it was only found at Elephant Mountain Wildlife Mountain Area in western Texas (Figure 3.7). This species also clustered in the *vexator* species group with *M. apache, M. californica,* and *M. vexator*.

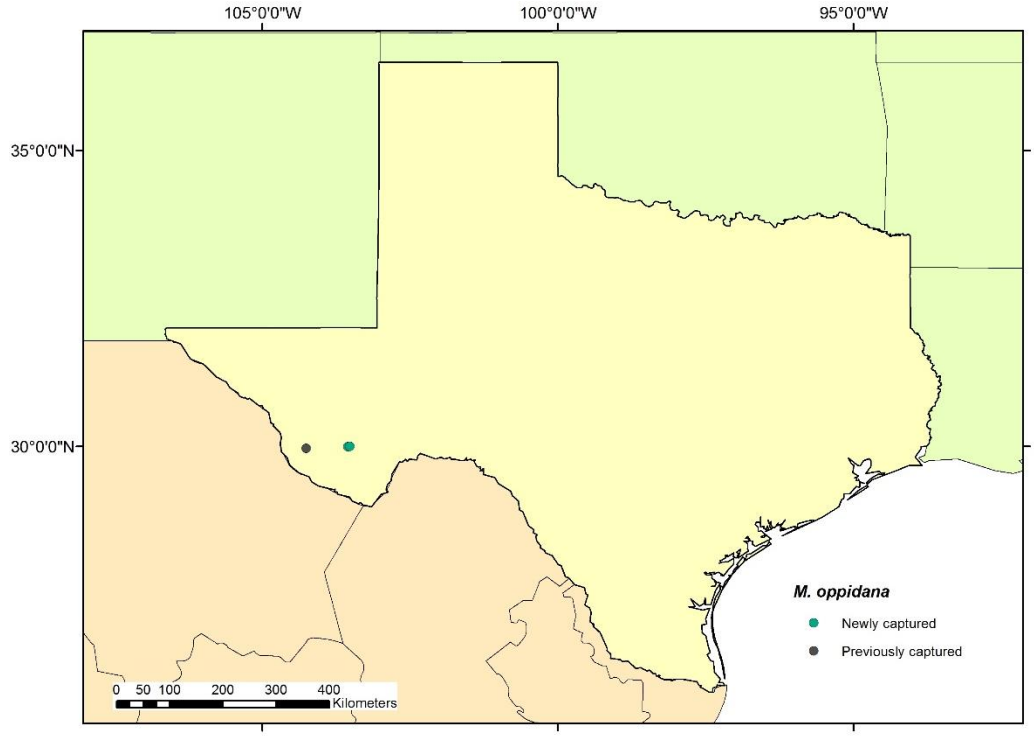

Figure 3.7: *Micropygomyia oppidana* captures are represented by turquoise points while past captures from Young and Perkins are denoted as gray points (Young and Perkins, 1984).

M. vexator is a pale and hairy fly ranging in size from 1.3 to 1.6 mm. It is widespread, ranging from Mexico to Canada and has been documented in 22 states from coast to coast (Claborn et al., 2009; Minter et al., 2009; Weng et al., 2012; Young and Perkins, 1984). This species is particularly interesting because morphological data suggests it has a subspecies called *M. vexator occidentis* with a western distribution. The Bayesian phylogenetic analysis suggested relatively deep evolutionary splits between *M. vexator* and its subspecies, *M. vexator occidentis.* These splits are even deeper than those of *M. vexator* with other *Micropygomyia.* All *M. vexator occidentis* and *M. vexator* identified morphologically and molecularly are displayed in Figure 3.8.

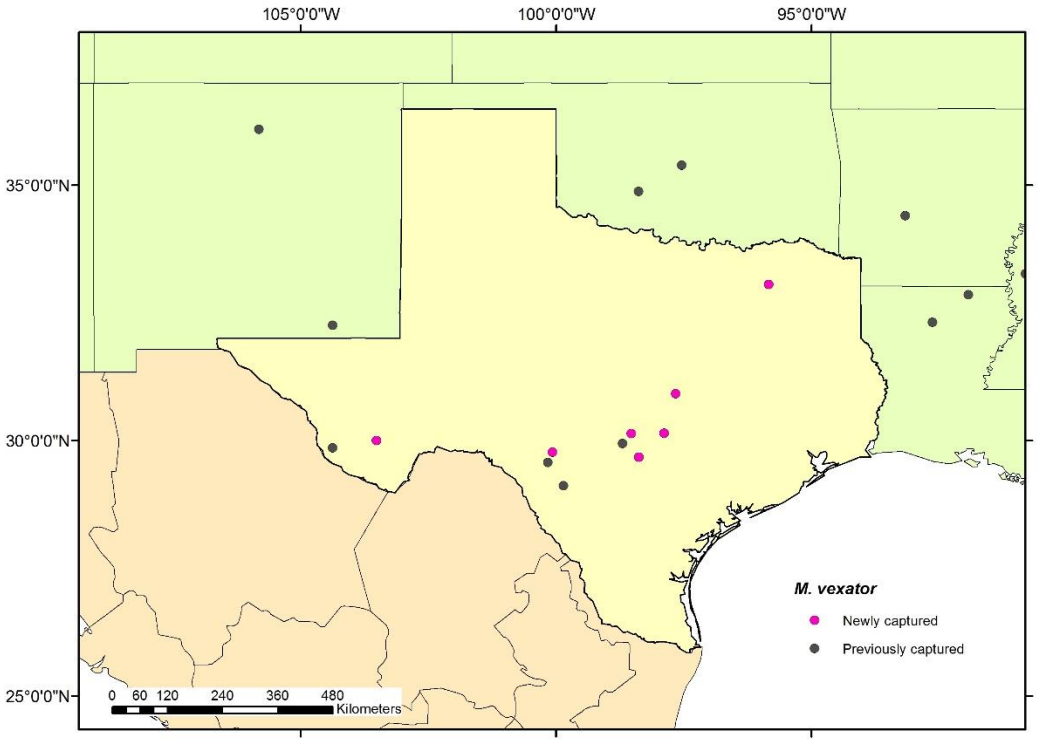

Figure 3.8: *Micropygomyia vexator* points are displayed in pink while previously captured flies are displayed in gray and taken from the Young and Perkins North American phlebotomine fly guide (Young and Perkins, 1984).

Psathromyia shannoni is a larger phlebotomine ranging across the entire continental United States (Figure 3.9) and into Texas and then southward to Argentina. Our study identified new specimens in the eastern portion of Texas, thus filling a gap between earlier captures in central Texas and those in Louisiana and Arkansas (Figure 3.9). *Ps. shannoni* individuals clustered together along one branch and appear to be a potential sister species to *Ps. texana*. Interestingly, these collections coincide with the recent range expansion of leishmaniasis into northern and eastern portions of Texas.

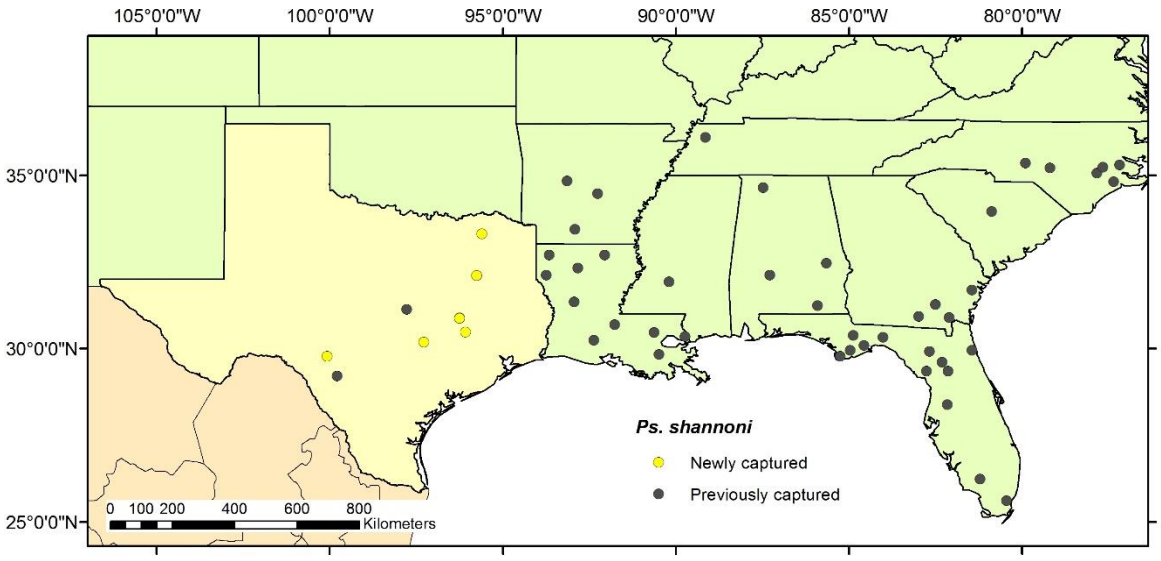

Figure 3.9: *Psathromyia shannoni* newly captured points are yellow while past captures based on the Young and Perkins North American guide are displayed in gray (Young and Perkins, 1984).

Psathromyia texana is a large and dark sand fly ranging in size from 1.8 to 2.1 mm distributed from Honduras to Texas (Christensen and de Vasquez, 1982; Young and Perkins, 1984). Within Texas, the species has been found in southern and central portions of the state before this study (Figure 3.10). I discovered this species just southeast of Dallas in Henderson county and in southeastern Texas at the Attwater Prairie Chicken National Wildlife Refuge and at two EcoLab sites. All these sites enlarge the known range of the species into eastern Texas where recent cases of leishmaniasis have been discovered.

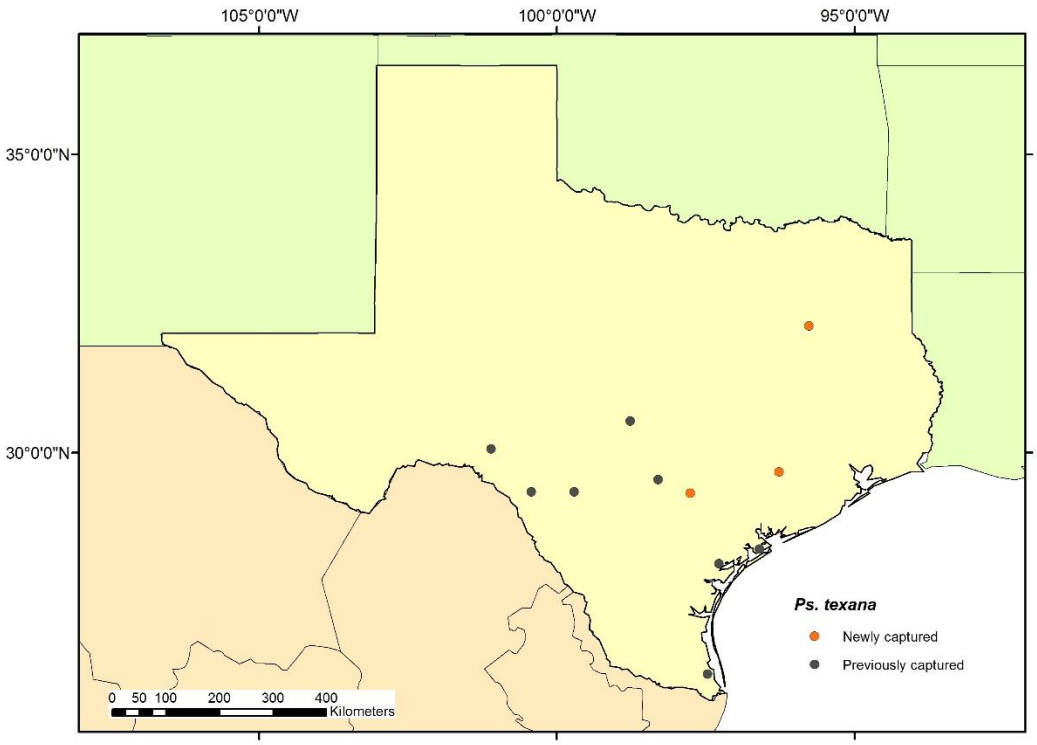

Figure 3.10: *Psathromyia texana* newly captured points are orange while past captures based on the Young and Perkins North American guide are displayed in gray (Young and Perkins, 1984).

Based on the barcoding analysis, only 4 species were clearly identified from this study's sampling across the state of Texas: *D. anthophora, M. diabolica. Ps. shannoni,* and *Ps. texana.* Specimens identified morphologically as *Micropygomyia apache, M. californica, M. oppidana,* and *M. vexator* clustered together based on the Bayesian phylogeny produced from the *cyt b* gene. I will, henceforth, refer to these 4 species as the *M. spp.* complex. Mapping this species group's distribution, shows that it is widespread across the western, central, and eastern portions of the state at approximately 30-33° latitude (Figure 3.11).

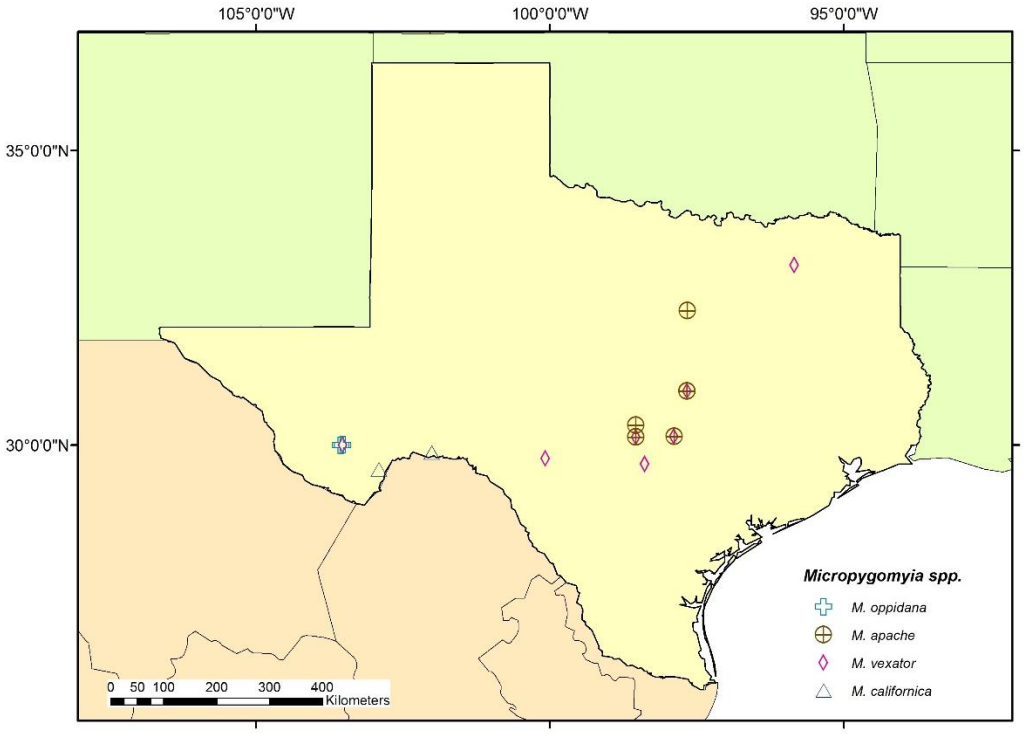

Figure 3.11: *M. spp.* identified via morphological means are displayed in separate colors: *M. oppidana* is represented by turquoise points, *M. apache* is represented by olive points, *M. vexator* is represented by pink points, and *M. californica* is represented by navy blue points.

DISCUSSION

Here, we found evidence for general range expansions in 4 species (*D. anthophora, Lu. diabolica, M. apache, and Ps. texana*) with evidence for northward shifts in at least 3 species (all but *M. apache*). Significantly, many of these phlebotomine species' enlarged distributions overlap with the recent expansion of leishmaniasis in Texas. Two of the four species with documented range increases meet the WHO criteria for being disease vectors (Table 3.2). This suggests that the recent expansion of leishmaniasis may in part be due to movement of the disease through these vectors. *Dampfomyia anthophora* meets 4 out of 5 WHO vector criteria and has been found further east and north than previously documented. *Lutzomyia diabolica* also shows increased range expansion into eastern and northern Texas than previously documented and meets 3 of 5 WHO vector criteria.

Previously, 8 out of 13 species of phlebotomine flies found in the United States were documented in Texas. Our study adds *M. apache* to this list. *Lutzomyia aquilonia* occurs in Texas (Claborn et al., 2009; Young and Perkins, 1984) but was not captured in this study. *Lutzomyia aquilonia* has only been captured at Fort Hood in Texas (Claborn et al., 2009) although based on the literature, it does have a fairly wide distribution ranging from the southern United States to Canada (Table 1).

This study has also increased information about *Psathromyia shannoni's* distribution filling a gap between records in the eastern United States and Texas. It also meets 3 of 5 WHO vector criteria. Although it has been recorded in Arkansas and Louisiana, it had not previously been recorded in East Texas. The shared distributional overlap of this species with *Lu. diabolica* and *D. anthophora* raises the concern that the parasite could be transmitted to this species when occurring in the same habitat and feeding on the same reservoirs. Comprehensive studies should be carried out to determine the feeding preferences of these various species to determine whether there is a risk of transmission into *Ps. shannoni* within Texas.

The new documentation of a larger range of *M. apache* is somewhat disconcerting as it has been a reported vector for vesicular stomatitis virus, a disease mainly found in agricultural animals with symptoms similar to Foot and Mouth disease (Reeves et al., 2008). *M. apache* has not yet been found infected with leishmaniasis.

In regards to the *M. spp.* clade, multiple specimens were identified as different species but clustered together on the same clade as a polytomy. This may, in part, be due to the use of only one mitochondrial molecular marker, *cyt b,* not giving enough resolution; therefore, the possibility remains that these are truly separate species but we were unable to detect them as such. Or these specimens may actually comprise a single species that has been incorrectly split via morphological methods. Another possibility is that a new species hybridized with these other species and contributed maternally to the haplotype. Several *M. vexator* specimens including *M. vexator occidentis* were identified within their own clade separately from the other *M. vexator* specimens identified morphologically.

Additionally, this group was very difficult to identify morphologically. Leading experts in the field of phlebotomine taxonomy made different identifications of the same specimens. Often, *M. apache* and *M. vexator* specimens were difficult to identify and found at the same sites and thus, reserved for molecular analysis. It is possible that *cyt b* isn't evolving quickly enough to distinguish the *M. spp.* from one another.

Overall, the barcoding analysis suggests major revisions to the taxonomy of phlebotomine flies in the United States and a potential new species has been identified. The analysis also documented multiple specimens collected beyond the previously known range limits of the species. Importantly, some of these range expansions occurred in the same regions of increased *Leishmania mexicana* activity. While this study did not explicitly examine biotic interactions between hosts and between phlebotomine flies, it is an important step in guiding future analyses.

Chapter 4: Leishmaniasis screening

ABSTRACT

After trapping cutaneous leishmaniasis vectors and reservoirs across Texas using a broad sampling scheme, specimens were tested. A leishmaniasis PCR assay using kinetoplast minicircle DNA was used and tested extensively for sensitivity. There are up to 10,000 minicircles per *Leishmania* parasite making this an extremely sensitive target. Phlebotomine flies infected for different periods of time (day 2, day 6, and day 8) and a kinetoplast cloned insert were used to examine sensitivity. Day 2 infections in phlebotomines were able to be detected while the cloned insert was able to be detected in as few as 10 copies. Pools of phlebotomines were tested from different sites. A single female *Dampfomyia anthophora* was found infected in Brazos county at a site that had both *Psathromyia shannoni,* a possible vector, and *Neotoma floridana,* a possible reservoir.

INTRODUCTION

Cutaneous leishmaniasis has been found across the southern United States as discussed in the first chapter. The causative agent of the cases in Arizona, Texas, and Oklahoma is *Leishmania mexicana.* While one locality of *Le. mexicana* has been recorded in Arizona, Texas and Oklahoma have undergone the most dramatic emergence. Therefore, this research project focuses on the Texas distribution.

Multiple species have been found infected in Texas with *Le. mexicana* ranging from suspected phlebotomine vectors to woodrat reservoirs to other mammalian species. It is unclear whether or not these other mammals play a major role as reservoirs in the enzootic cycle. The pathogen has also been documented in humans, dogs, and domestic cats.

In order to understand the complex enzootic cycle of the parasite, it is necessary to first discover where the pathogen occurs and in what organisms. The location of the parasite indicates two key pieces of information: what habitat is preferred and if the

pathogen has expanded its range. The organisms found infected tell us what the potential vectors and reservoirs might be and whether there has been a host shift into new species.

This study aimed to develop and validate tools for screening for leishmaniasis in vectors and reservoirs in addition to discovering where in Texas leishmaniasis is presently and if it is occurring in areas predicted by the species distribution modeling in chapter 2. Additionally, this particular phase of the project is a key first step in understanding the eco-epidemiology of the parasite by discerning what potential and new hosts may be infected and whether those hosts should undergo further analysis in regards to their vector and reservoir status.

METHODS

Phlebotomine flies were sampled following the procedures outlined in chapter 3. Rodents were collected and handled in accordance to the guidelines of the American Society of Mammalogists Animal Care and Use Committee, specifically protocol AUP-2013-00040. Each collecting site was surveyed using opportunistic trapping for 1-2 trap nights. Sherman live traps (folding aluminum traps) were placed in the evening/dusk and collected at dawn before temperatures rose. All traps were given a site number, GPS coordinates, and surveying tape to aid in faster trap recovery. Traps were baited and left open throughout the night. For sites with high ant densities, Carbaryl powder was placed around and under our traps as suggested in the 2010 Kraig paper (Kraig et al., 2010). Carbaryl powder has commonly been used to repel flies, ticks, and fleas in livestock and pets and has low toxicity to mammals.

Empty traps were closed in the morning and occupied traps were processed at a mobile field processing station within walking distance to all trap lines. All animals were processed individually. Individuals handling animals wore disposable latex/nitrile gloves under leather/mechanic gloves if needed. While handling species known to be potential disease reservoirs, goggles and a respirator or face shield were used in addition to disposable aprons and/or sleeve covers. After processing, animals were placed into a clear

plastic observation chamber and/or the original Sherman Trap and then released at their respective trapping locations after the completion of all processing.

Captured animals were anesthetized for several minimally invasive procedures using isoflurane. The present ecological field study required a simple, efficient, and short-term (<7 minutes) anesthetic for sampling small mammals that cannot be transported to a central processing facility. We preferred to process them in the field and release them as soon as they recovered. Anesthesia was maintained using a nose cone constructed of a 50ml centrifuge tube with cotton/gauze slightly moistened (<1 ml) with the 20% isoflurane mixture pushed to the bottom of the tube or undiluted isoflurane as opposed to methoxyflurane (Itah et al., 2004; Parker et al., 2008).

While under anesthesia, all animals were identified using taxonomic keys. Standard measurements including weight, total length, tail length, hindfoot length, and ear length were recorded. While under anesthesia, ectoparasites such as fleas, ticks, and mites were removed and animals were fitted with an ear tag (National Band and Tag Company, Newport, KY). This acted as a safe guard against taking blood samples twice in the same 24 hour period and allowed us to determine whether or not an animal had been trapped in previous years. Blood was collected for a collaborative project on Plague distribution with the Texas Department of State Health Services but was not relevant to this study. Two ear punches were taken for leishmaniasis testing. We only tested rodents at sites where phlebotomine flies were found infected due to time restrictions.

To screen for leishmaniasis, a *Leishmania mexicana* kinetoplast DNA (kDNA) target was selected. The kinetoplast is a large organelle similar to a mitochondrion that powers the flagellum of the *Leishmania* parasite. Within the kinetoplast there are maxicircles and minicircles, which are configured as a network of circularized DNA molecules (Shlomai, 2004). We chose this approach as there are up to 10,000 minicircles per parasite (Rogers and Wirth, 1987) making this an extremely sensitive screening test shown to have sensitivities of 98.7% identifying 77/78 infections (Bensoussan et al., 2006).

Previously developed primers developed were used to amplify a 700 bp region of the kDNA: 5'- CTR GGG GTT GGT GTA AAA TAG – 3' (L.MC-1S), and 5' – TWT

GAA CGG GRT TTC TC – 3' (L. MC-1R) (Kato et al., 2005). To validate the ability of the primers to detect phlebotomine infections, were tested on phlebotomine flies that were experimentally infected with *Le. mexicana* at different time points (2, 6, and 8 days) (Figure 4.1) obtained from the National Institutes of Health Laboratory of Malaria and Vector Research. This particular strain was isolated from a 30 year old male in Mexico in 1984 (MH0M/MX/84/SET GS). Day 6 and day 8 were clearly detected while Day 2 was weakly detected. The Day 2 and Day 8 infections were used as positive controls in subsequent stages of testing.

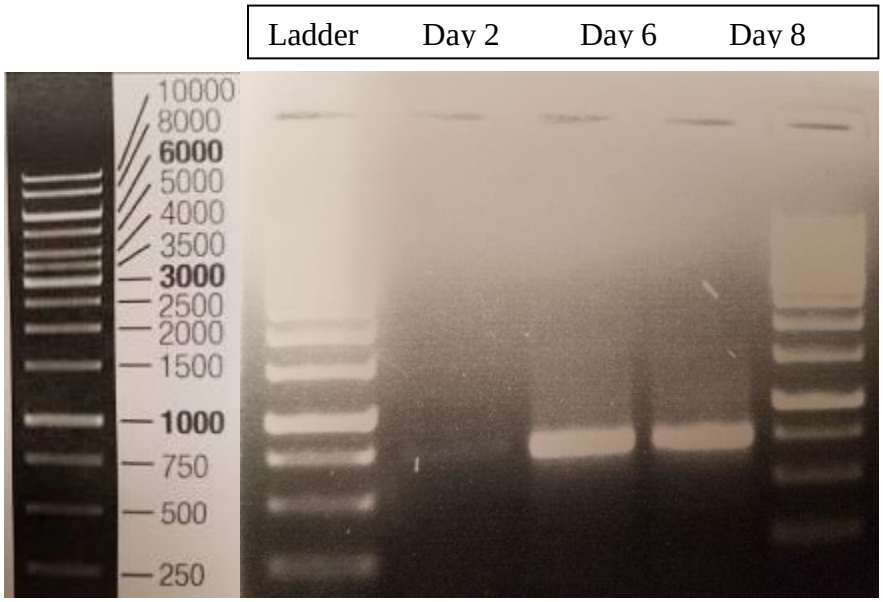

Figure 4.1: Day 2, 6, and 8 *Le. mexicana* infections of phlebotomine flies obtained from NIH.

In order to screen for leishmaniasis and assess the sensitivity of our PCR-based assay, an additional positive control was created using the StrataClone PCR cloning kit. The L. MC-1S and L. MC-1R primers were used to first amplify a 700 bp region of DNA from a *Le. mexicana* day 8 infected sand fly. This product was then inserted into the StrataClone Solo Pack E. coli plasmid. After insertion and culturing, a restriction digest using EcoR1 was carried out to discern whether or not the kDNA minicircle insert had actually been integrated in the plasmid. The digest product was run on a gel and a band of ~700 bp was detected along with the remaining plasmid DNA (Fig 4.2). Lastly, the identity

of the cloned product was confirmed by Sanger sequencing. A BLAST search of the resulting sequence most closely aligned with a complete unpublished sequence of *Le. mexicana* kinetoplast minicircle DNA entry from GenBank: AY145437.

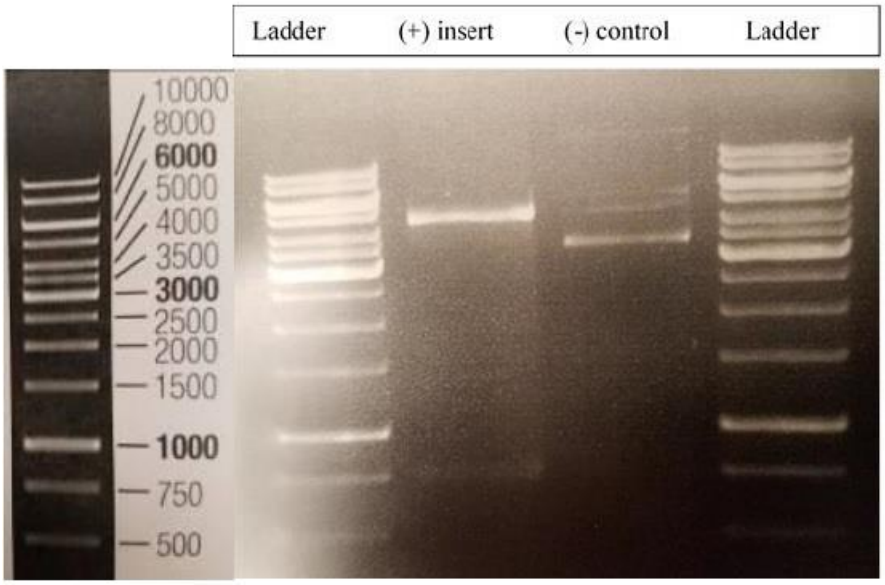

Figure 4.2: Restriction Digest of *E. coli* plasmid with *Le. mexicana* gene and negative control.

The detection limit was determined via serial dilutions of the plasmid and the day 8 infected phlebotomine fly. The initial DNA extract from the plasmid was 51.6 ng/µl and after a serial dilution, was less than 5.16×10^{-8} ng/µl. Even this lowest dilution, corresponding to approximately 10 copies of the plasmid, was able to be detected, while negative controls lacking template DNA remained negative (Figure 4.3). Moreover, *Le. mexicana* kDNA was able to be detected from Day 8 infected phlebotomine flies after a 1:100 fold dilution of an initial concentration of 1.7 ng/µl. Thus the test is extremely sensitive, and negative results likely reflect an absence of infection (Figure 4.4).

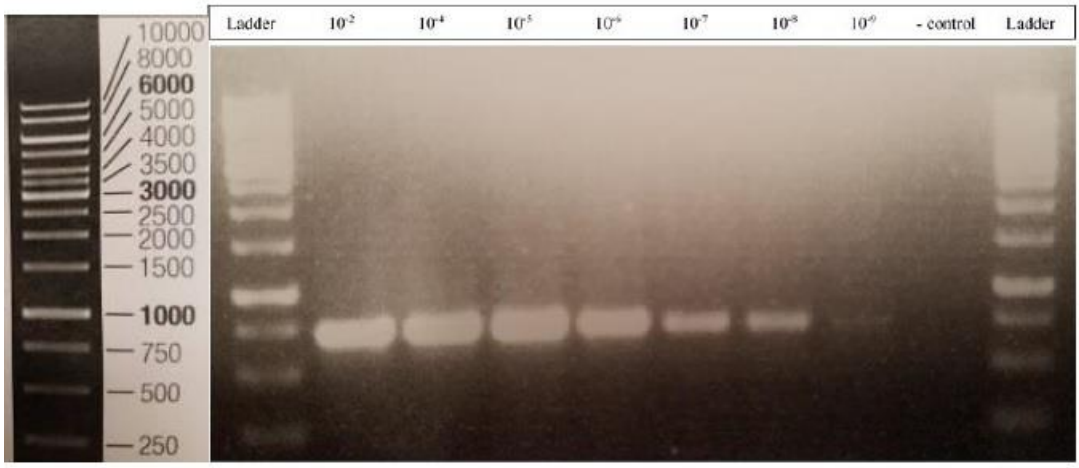

Figure 4.3: Dilutions of positive control plasmid insert.

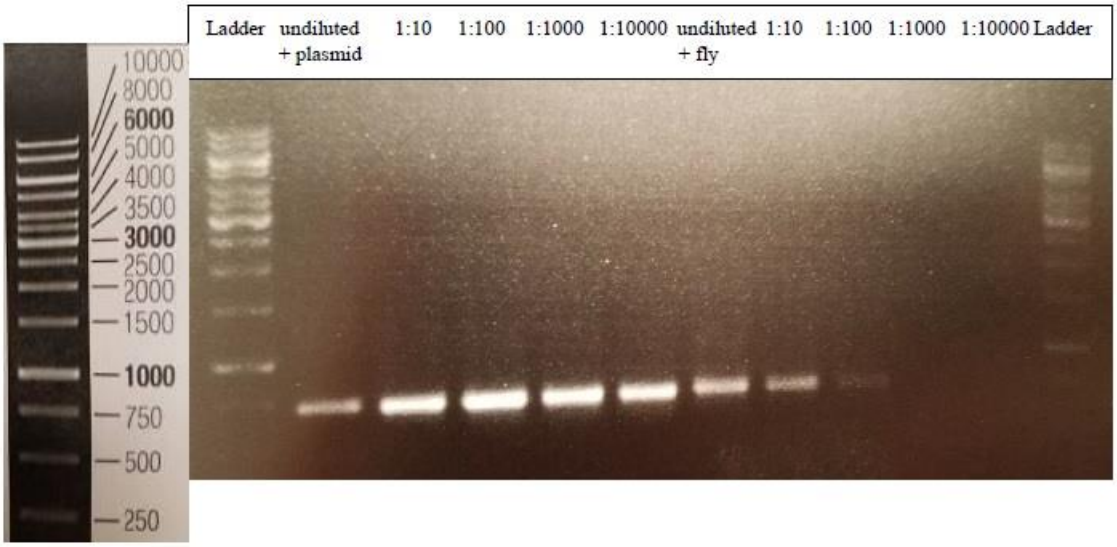

Figure 4.4: Dilutions of plasmid positive control and infected phlebotomine positive control.

DNA extraction was carried out using Qiagen DNeasy blood and tissue kits with minor modifications. The final elution step resulted in 75 µl of material as opposed to 200 µl in order to produce a more concentrated DNA sample for each individual fly.

Female phlebotomine flies were subsampled for identification due to the large number collected between 2013 and 2015. In order to reduce costs and make testing more

efficient, flies were pooled according to site and the subsample of species identified. A total of 120 flies were grouped into 49 pools for testing (Table 4.1, Table 4.2). For PCR, 0.5 – 2.0 µl of the DNA extract from each fly based on DNA concentration. This method was used so that there would not be too much DNA in each PCR reaction thus inhibiting the reaction.

DNA concentration (µl/ng)	Volume used for PCR reaction
0.0 – 2.0	2 µl
≥ 2.0 – 5.0	1 µl
≥ 5.0	0.5 µl

Table 4.1: Volumes based on DNA concentration used for PCR.

The total volume for each PCR reaction was 25 µl with 12.5 µl of Promega GoTaq master mix (add in company) and after the pooled volumes were added ranging from 0.5 µl to 11 µl. The largest site had 11 phlebotomine flies originating from the Matador Wildlife Management Area while several sites consisted of only 1 phlebotomine fly (Table 3).

At sites of positive *Le. mexicana* results, rodents were also tested. However, rodent trapping was discontinued in 2014 due to the lack of rodents being captured and lack of time and assistance available for trapping.

Pool	Year	Site	*Species*	Accession Number	DNA Conc. (ul/ng)	PCR Vol. (ul)	Total DNA (ng)	Total Pool Vol. (ul)
1	2013	Siebert	*Lutzomyia diabolica*	A1_1_2013	9.7	0.5	4.85	0.5
2	2013	Bastrop 4	*Lu. diabolica*	A16_1_2013	10.0	0.5	5.00	0.5
3	2013	Attwater Prairie Chicken Reserve	*Psathromyia texana*	A2_1_2013	2.6	1.0	2.60	1.0
4	2013	Blanco 10	*Micropygomyia apache*	A19_1_2013	6.0	0.5	3.00	1.0
				A30_1_2013	8.6	0.5	4.30	
5	2013	Blanco 10	*Lu. diabolica*	A29_1_2013	6.4	0.5	3.20	0.5
6	2013	Bell 1	*M.apache*	A35_1_2013	5.7	0.5	2.85	0.5
7	2013	Bell 1	*M. vexator*	A37_1_2013	4.0	1.0	4.00	1.0
8	2013	Brazos 2	*Dampfomyia anthophora*	A40_1_2013	2.8	1.0	2.80	2.0
				A41_1_2013	2.7	1.0	2.70	
9	2013	Brazos 2	*P. shannoni*	A47_1_2013	3.1	1.0	3.10	2.0
				A48_1_2013	3.5	1.0	3.50	
10	2013	Bastrop 9	*D. anthophora*	A15_1_2013	1.2	2.0	2.40	2.0
11	2013	Bastrop 9	*P. shannoni*	A51_1_2013	4.3	1.0	4.30	1.0
12	2013	Henderson1	*P. shannoni*	A55_1_2013	3.6	1.0	3.60	1.0
13	2013	Henderson1	*P. texana*	A57_1_2013	3.9	1.0	3.90	1.0
14	2013	Guadalupe 1	*P. texana*	A58_1_2013	2.2	1.0	2.20	1.0
15	2013	Hays 7	*Lu. diabolica*	A63_1_2013	2.7	1.0	2.70	3.5
				A63_2_2013	5.0	0.5	2.50	
				A65_1_2013	2.7	1.0	2.70	
				A65_2_2013	4.4	1.0	4.40	
16	2014	Bandera 5	*Lu. diabolica*	B4_1_2014	2.6	1.0	2.60	1.0
17	2014	Merkord	*M. californica*	B15_1_2014	4.6	1.0	4.60	1.0
18	2014	Bastrop 9	*Lu. diabolica*	B16_1_2014	11.5	0.5	5.75	0.5
19		Matador WMA	*Lu. diabolica*	B17_1_2014	2.5	1.0	2.50	11.0
				B18_1_2014	2.7	1.0	2.70	
				B19_1_2014	3.1	1.0	3.10	
				B5_1_2014	2.4	1.0	2.40	
				B5_2_2014	2.4	1.0	2.40	
				B5_3_2014	2.0	1.0	2.00	
				B5_4_2014	3.2	1.0	3.20	
				B5_5_2014	2.9	1.0	2.90	
				B5_6_2014	2.3	1.0	2.30	
				B5_7_2014	2.7	1.0	2.70	
				B28_1_2014	2.0	1.0	2.00	

Table 4.2

20	2014	Harlingen	*D. anthophora*	B31_1_2014	2.6	1.0	2.60	3.0
				B31_3_2014	2.6	1.0	2.60	
				B32_2_2014	3.9	1.0	3.90	
21	2014	Blanco 10	*Lu. diabolica*	B34_1_2014	6.0	0.5	3.00	1.5
				B34_2_2014	2.5	1.0	2.50	
22	2014	Hopkins 1	*M. vexator occidentis*	B37_1_2014	3.3	1.0	3.30	1.0
23	2014	Hopkins 1	*Lu. diabolica*	B57_1_2014	2.8	1.0	2.80	1.0
24	2014	Bandera 11	*Lu. diabolica*	B38_1_2014	2.8	1.0	2.80	1.0
25	2014	Taylor Unit Playa Lakes WMA	*D. anthophora*	B52_1_2014	3.5	1.0	3.50	1.0
26	2014	Real 5	*D. anthophora*	B56_1_2014	2.3	1.0	2.30	1.0
27	2014	Real 5	*Lu. diabolica*	B58_1_2014	10.2	0.5	5.10	1.5
				B61_2_2014	11.4	0.5	5.70	
				B64_1_2014	10.2	0.5	5.10	
28	2013	Edwards 5	*D. anthophora*	C1_1_2013	3.5	1.0	3.50	2.0
				C1_4_2013	2.8	1.0	2.80	
29	2013	Edwards 5	*Lu. diabolica*	C1_10_2013	3.2	1.0	3.20	5.0
				C1_2_2013	3.0	1.0	3.00	
				C1_3_2013	3.3	1.0	3.30	
				C1_5_2013	3.5	1.0	3.50	
				C1_6_2013	3.2	1.0	3.20	
30	2013	Edwards 5	*P. shannoni*	C1_7_2013	3.4	1.0	3.40	1.0
31	2014	Edwards 5	*Lu. diabolica*	C2_1_2014	3.2	1.0	3.20	9.5
				C2_2_2014	3.1	1.0	3.10	
				C2_3_2014	6.4	0.5	3.20	
				C2_4_2014	3.5	1.0	3.50	
				C2_5_2014	2.6	1.0	2.60	
				C2_6_2014	2.5	1.0	2.50	
				C2_7_2014	3.1	1.0	3.10	
				C2_8_2014	2.5	1.0	2.50	
				C2_9_2014	4.2	1.0	4.20	
				B14_1_2014	2.3	1.0	2.30	

Table 4.2

32	2014	Bandera 5	*Lu. diabolica*	C3_1_2014	2.9	1.0	2.90	9.5
				C3_2_2014	3.0	1.0	3.00	
				C3_3_2014	3.1	1.0	3.10	
				C3_4_2014	2.8	1.0	2.80	
				C3_5_2014	3.6	1.0	3.60	
				C3_6_2014	1.8	2.0	3.60	
				C3_7_2014	4.2	1.0	4.20	
				C3_8_2014	3.8	1.0	3.80	
				C3_9_2014	6.4	0.5	3.20	
33	2014	Gillespie 1	*Lu. diabolica*	C4_1_2014	3.8	1.0	3.80	6.0
				C4_2_2014	2.8	1.0	2.80	
				C4_3_2014	3.1	1.0	3.10	
				C4_4_2014	2.4	1.0	2.40	
				C4_5_2014	2.8	1.0	2.80	
				C4_7_2014	2.6	1.0	2.60	
34	2013	Bexar 3	*Lu. diabolica*	C5_1_2013	4.7	1.0	4.70	6.0
				C5_2_2013	3.6	1.0	3.60	
				C5_3_2013	3.5	1.0	3.50	
				C5_4_2013	3.2	1.0	3.20	
				C5_6_2013	3.1	1.0	3.10	
				C5_7_2013	2.7	1.0	2.70	
35	2013	Bexar 3	*M. vexator*	C5_8_2013	3.0	1.0	3.00	1.0
36	2014	Bastrop 4	*Lu. diabolica*	C6_1_2014	4.3	1.0	4.30	6.0
				C6_2_2014	3.1	1.0	3.10	
				C6_3_2014	2.2	1.0	2.20	
				C6_4_2014	3.7	1.0	3.70	
				C6_5_2014	3.4	1.0	3.40	
				C6_6_2014	3.5	1.0	3.50	
37	2015	Travis 30	*Lu. diabolica*	D11_1_2015	6.4	0.5	3.20	0.5
38	2015	Madden Prairie Reserve	*Lu. diabolica*	D15_1_2015	3.0	1.0	3.00	2.0
				D15_2_2015	2.4	1.0	2.40	
39	2015	Travis/Hays 1	*Lu. diabolica*	D24_1_2015	13.9	0.5	6.95	3.0
				D26_1_2015	16.2	0.5	8.10	
				D27_1_2015	3.9	1.0	3.90	
				D28_1_2015	12.8	0.5	6.40	
				D30_1_2015	17.7	0.5	8.85	
40	2015	Somervell 1	*M. apache*	D31_1_2015	3.9	1.0	3.90	1.0

Table 4.2

41	2015	Somervell 1	*D. anthophora*	D31_2_2015	3.5	1.0	3.50	1.0
42	2015	Cooper WMA	*P. shannoni*	D39_1_2015	2.9	1.0	2.90	1.0
43	2015	Travis 24	*Lu. diabolica*	D60_1_2015	5.2	0.5	2.60	2.0
				D60_2_2015	7.2	0.5	3.60	
				D47_1_2015	7.1	0.5	3.55	
				D61_1_2015	7.8	0.5	3.90	
44	2015	Atascosa 1	*D. anthophora*	D52_1_2015	4.9	1.0	4.90	2.0
				D52_2_2015	3.1	1.0	3.10	
45	2014	Edwards 4	*Lu. diabolica*	D55_1_2014	7.2	0.5	3.60	1.5
				D55_2_2014	7.1	0.5	3.55	
				D55_3_2014	11.1	0.5	5.55	
46	2015	Travis 32	*Lu. diabolica*	D58_2_2015	6.0	0.5	3.00	1.0
				D58_4_2015	5.1	0.5	2.55	
47	2015	Elephant Mountain WMA	oppidana	D62_1_2015	15.6	0.5	7.80	0.5
48	2015	Elephant Mountain WMA	*M. spp.* (not identifiable to species level)	D63_1_2015	5.9	0.5	2.95	1.0
				D46_1_2015	5.4	0.5	2.70	
49	2015	Hays 12	*M. vexator*	D8_1_2015	2.0	1.0	2.00	1.0

Table 4.2: Pools determined by site and species collected.

RESULTS

Of the 49 pooed samples of phlebotomine flies tested, only site 8 (the Brazos 2 site) tested positive (Figures 4.5, 4.6, 4.7). This pool came from a sample of two *Dampfomyia anthophora* phlebotomine flies collected in Brazos County, Texas, on August 20, 2013.

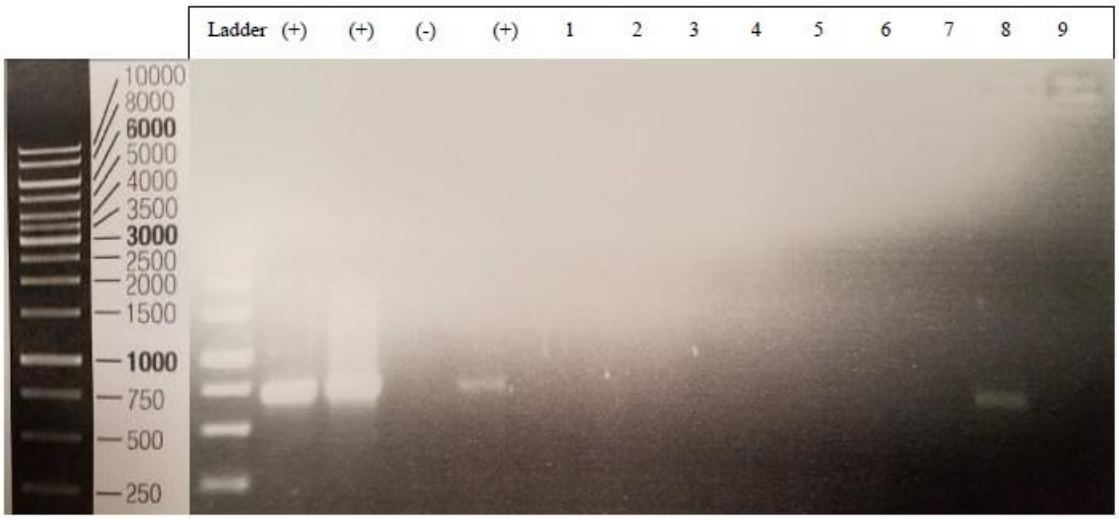

Figure 4.5: Positive and negative controls and pools 1-9. The 2nd lane consisted of the plasmid at a 1:1,000,000 dilution, the 3rd lane was a day 8 infected fly, and the 5th pool represented a day 2 infected fly. Pool 8 (site Brazos 2) tested positive.

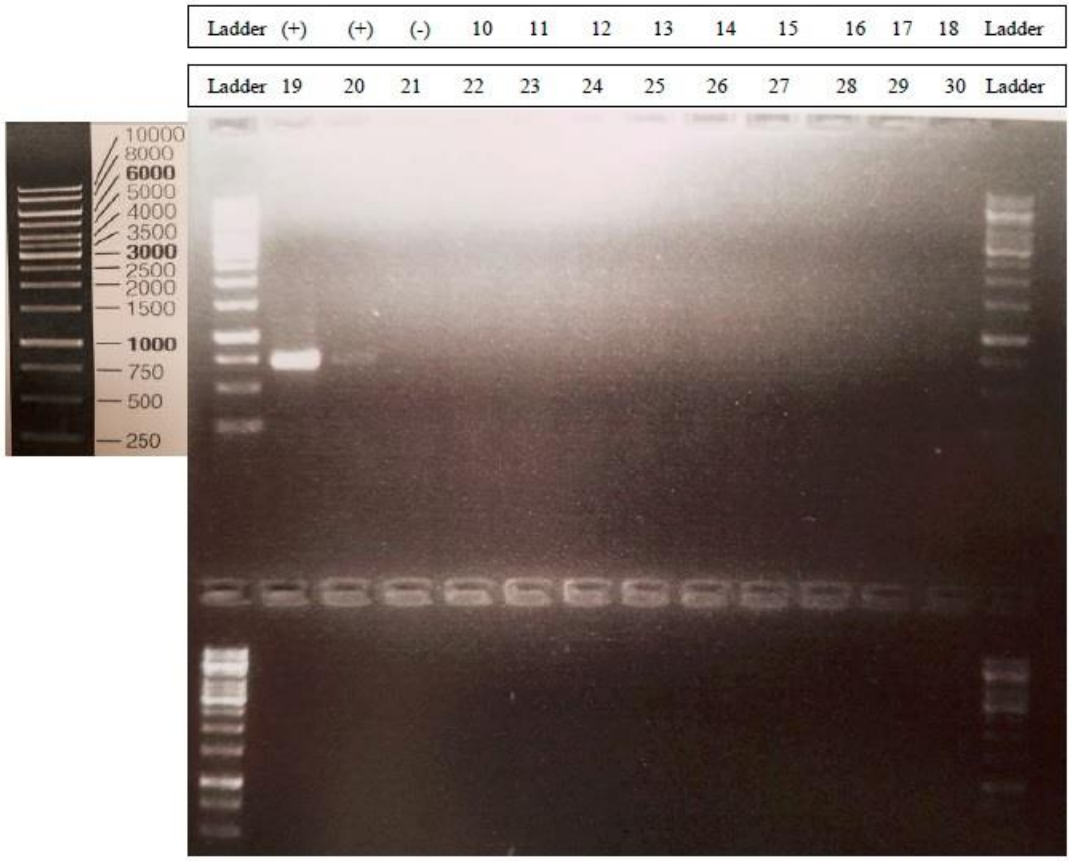

Figure 4.6: Positive controls (lane 2: 1:1,000,000 plasmid insert, lane 3: day 2 infected fly), negative control (lane 4) and samples 10-30 tested.

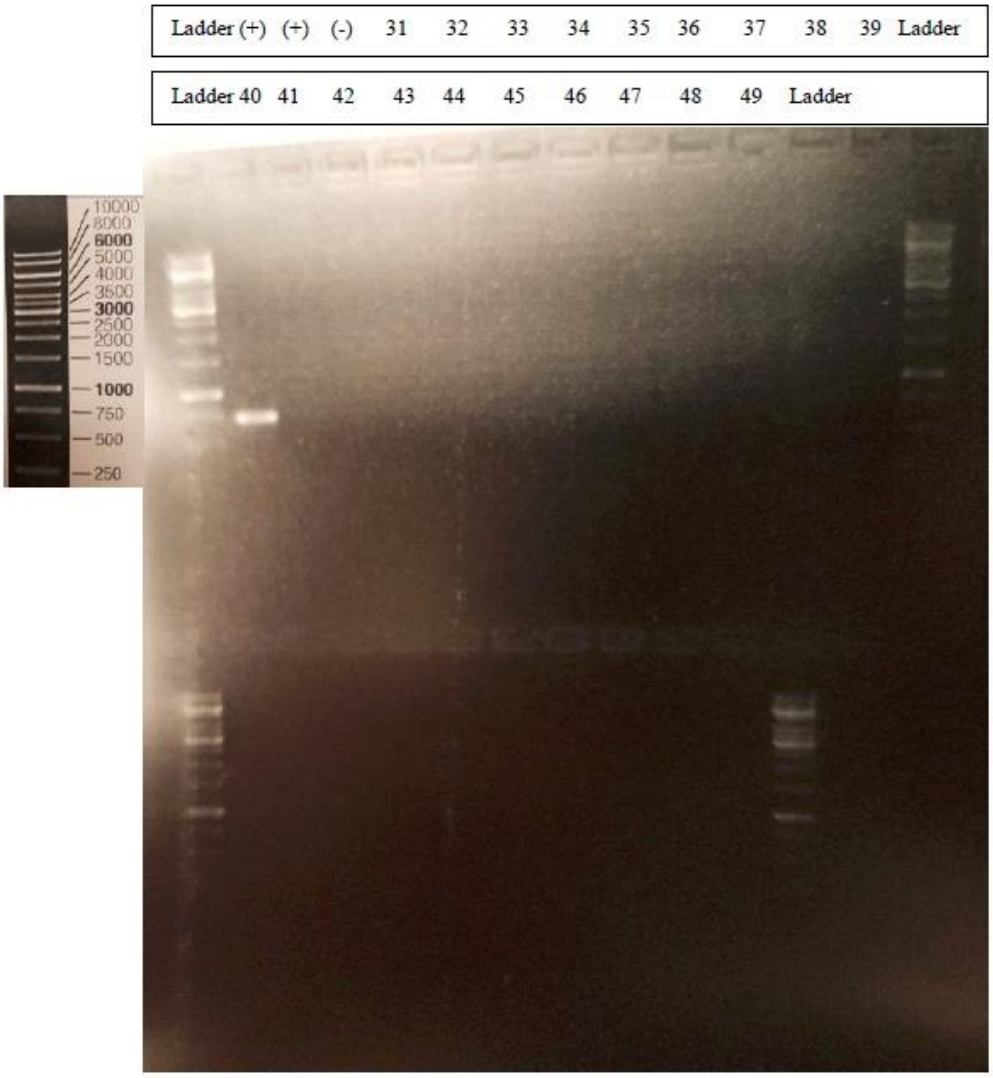

Figure 4.7: Positive controls (lane 2: 1:1,000,000 plasmid insert, lane 3: day 2 infected fly), negative control (lane 4) and samples 31-49 tested. All tests came back negative. The lane 3 infected day 2 fly is difficult to make out in this image but can be seen in another close up image although very faintly.

After obtaining a positive result, each individual in the pool was tested separately. One individual tested positive (Figure 4.8). Rodents were also caught at this site: two *Neotoma floridana* and one *Peromyscus leucopus*. One of the *N. floridana* escaped and we were unable to obtain ear punches for testing. Both remaining rodents tested negative for *Le. mexicana*. The remaining 48 pools of phlebotomines tested negative for *Leishmania*

mexicana. Previous sites of infection of *D. anthophora* and *N. floridana* are shown in Figure 4.9 along with the newly discovered case in *D. anthophora* at the Brazos 2 site.

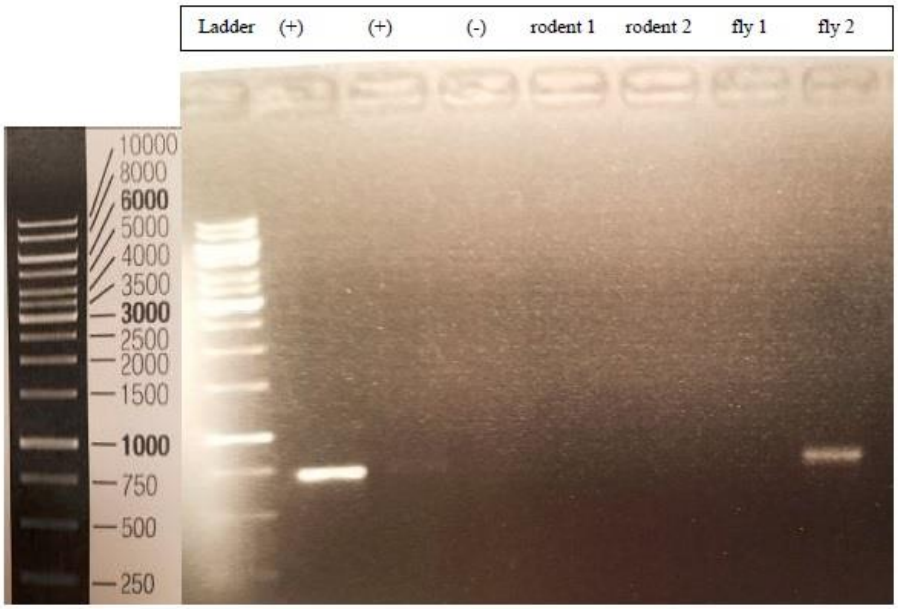

Figure 4.8: Lanes 2 and 3 served as positive controls (lane 2: 1:1,000,000 plasmid insert, lane 3: day 2 infected fly) while lane 4 was the negative control. The first rodent in lane 5 was *Peromyscus leucopus,* the second rodent was *Neotoma floridana*—both tested negative for *Le. mexicana.* The second phlebotomine fly (lane 8) tested positive for the parasite but not the first fly.

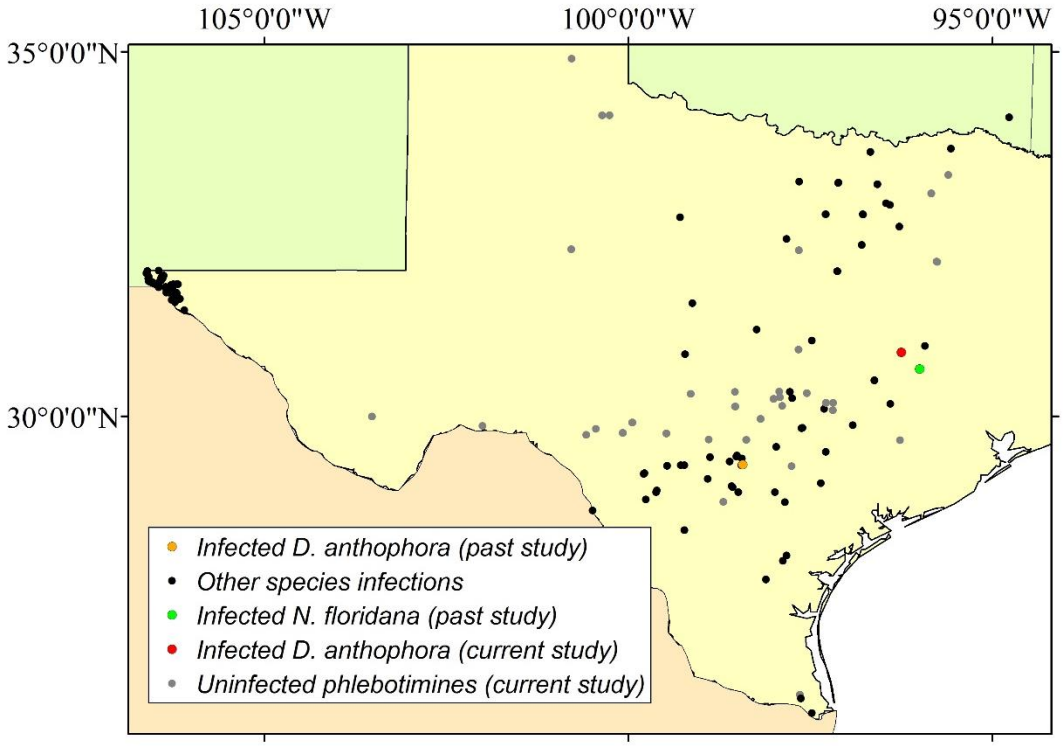

Figure 4.9: Black points represent previously documented isolations of *Le. mexicana* or cases of cutaneous leishmaniasis. Gray points represent collection sites for this paper of phlebotomine flies and/or rodents. The orange point represents a previously documented isolation of the parasite in *Dampfomyia anthophora* while the red point represents the current isolation. The green point represents the isolation of the parasite from a potential new woodrat reservoir host, *Neotoma floridana,* found in a previous study.

DISCUSSION

Le. mexicana was found in one female *Dampfomyia anthophora* phlebotomine fly in Brazos county. This species meets 4 of 5 WHO vector criteria and based on sampling for this study was documented substantially further north and east than it had been documented before (chapter 3).

Another species sampled at the Brazos 2 site, *Psathromyia shannoni,* was not found infected. This is of particular interest because this species meets 4 of 5 vector criteria as well, similar to *D. anthophora*. *Psathromyia shannoni* ranges eastward all the way to the Atlantic coast and occupies different habitat than both *Lu. diabolica* and *D. anthophora;* in particular, forests and areas with greater precipitation. If the parasite has jumped into a

new species of phlebotomine fly, such as *P. shannoni,* this could mean expansion of the disease much further north and east than previously documented and would dramatically increase risk.

The fact that these two phlebotomine fly species (*D. anthophora* and *P. shannoni)* occur in syntopy, the occurrence of two species in the same habitat at the same time, is somewhat concerning given that my previous analysis in chapter 1 suggests *P. shannoni* to be a competent vector. *P. shannoni* is documented at a site where there is the pathogen that causes cutaneous leishmaniasis. This distributional overlap opens the possibility of a real species shift from one vector, *D. anthophora,* to another, *P. shannoni*. And in fact, this may have already occurred.

Surprisingly, no *Lu. diabolica* flies tested positive despite it being the far more abundant species collected and a suspected vector. No *Micropygomyia spp.* or *P. texana* were found infected. These species had not previously been implicated in the enzootic Texas/Oklahoma cycle.

The rodents, *P. leucopus* and *N. floridana,* collected at the site of the positively infected phlebotomine fly both tested negative. However, this should not be interpreted as a definitive negative site given the small sample size. Additionally, *N. floridana* is the only known woodrat species found in this particular region of Texas. Because the parasite required both a vector and reservoir host species to carry out its life cycle, this woodrat species is the likely reservoir. This is further evidence of a potential host shift into a new woodrat species. The expansion into a new woodrat species should not be treated lightly as this opens up the possibility to expansion of the disease into the eastern United States.

Chapter 5: Conclusion

Climate change has and will continue to have dramatic effects on ecosystems. When discussing the effects of climate change on species, pathogens and their carriers are often overlooked. This may be due to a lack of data stemming from human privacy restrictions and human health concerns. The non-charismatic nature of these parasites and their vectors and reservoirs also leads to less collection and documentation. Vector-borne diseases are particularly poised for geographic range shifts, expansions, and contractions due to climate change.

One vector-borne disease, cutaneous leishmaniasis, has been emerging and re-emerging at various locations around the globe. Within the southern United States, this pathogen appeared to be shifting northward in congruence with our expectations of climate change. It has been emerging in areas northeastward of its earlier range without an explanation. It was speculated upon in the literature but no actual analyses of potential factors contributing to its expansion were analyzed. My aim was to examine whether or not climate change had contributed to the emergence of the parasite in northern and eastern Texas.

In order to examine the question of whether or not climate change had contributed to leishmaniasis expansion, a broad overview of the literature combined with data from the Texas Department of State Health Services was aggregated to determine the geographical extent of human and animal cutaneous leishmaniasis infections caused by *Le. mexicana*. The goal of the introductory chapter was to synthesize all previous knowledge of cutaneous leishmaniasis in the United States. Initially, only 42 human cases were documented in the literature (Clarke et al., 2013). After analyzing the literature and the Texas Department of State Health Services data, this number increased to 64 human cases between 1903 and 2015, many of which were recirculating within the past ranges of the 2000s and expanding north and east. However, the cases recirculating were only occurring in central and north Texas as opposed to the previous southern range from 1903-1989. In the 1990s the disease

moved north beyond the Edwards Plateau and began to spread northeastward in the 2000s to southeastern Oklahoma.

When this disease began occurring in new regions where it had never before been documented outside of the previous range of its suspected reservoir and vectors, multiple researchers wondered if it had jumped into new species. There was a lack of information on what species were infected because no widespread surveys in Texas had been carried out. Based on examination of the literature and recent studies carried out at the University of Texas at El Paso, a wide variety of sylvatic mammals were implicated in the zoonotic cycle.

An additional analysis of vector and reservoir criteria according to the World Health Organization was used to determine what other species could be vectors and reservoirs. Four species out of nine that occur in Texas were found to meet three or more vector criteria of the World Health Organization. One in particular, *Psathromyia shannoni,* had never before been implicated as a vector in the United States. This study determined that it meets four out of five WHO criteria and its range overlaps with the newly emerging foci of the disease.

From the database of the occurrence data of leishmaniasis infected organisms, the effect to which climate change had affected the geographical range of leishmaniasis in the southern United States was examined via species distribution modeling and a principal components analysis. While there are many studies examining the effect of climate change on ranges of vector-borne diseases in the future, there are few studies that have shown climate change to already have contributed to vector-borne disease expansion. Species distribution models were built forward and backward in time between two time periods associated with increased leishmaniasis activity. The model backcasted in time from the 2000s to the 1980s that used recent literature and Texas Department of State Health Services data was able to capture the past geographical range of the disease even though it overestimated disease risk in central Texas. This may have been due to a dispersal lag when the parasite spread to new hospitable areas. Both the forecasted and backcasted models showed northward movement of the range of the parasite.

Texas has been getting wetter since the 1980s and annual precipitation was one of the highest loading variables in the PCA and top contributing variable to the backcasted Maxent model. This makes sense in terms of the recent northeastern spread as the areas it is spreading to in central and eastern Texas are overall becoming more wet. In areas that are drier like south Texas, the parasite has not been seen recirculating.

In order to determine whether or not the climate space of the parasite had shifted geographically or the parasite's climate space preferences had changed, a principal components analysis was performed. Did the parasite experience an expansion or contraction of its niche or did its niche simply move in geographical space but remain the same? The PCA gave mixed results supporting both possibilities. There was overlap in climate space between the past and present disease cases. However, they also occupied different climate space. These results suggest that climate change has caused present day northeastern Texas to become more similar to past south Texas but also that the parasite may have shifted its host preference into a new reservoir or vector. Climate change may have paved the way for a species jump by bringing previously geographically separated species into contact with one another thus allowing the parasite to more easily jump into a new host species.

While performing the species distribution modeling and PCA, I realized there was a lack of spatially unbiased occurrence data available. Due to this lack of geographical and temporal occurrence vector, reservoir, and parasite data, I designed a broad sampling scheme across the state of Texas. When identifying vector species morphologically, multiple issues were encountered: damaged specimens, incomplete specimens, and ambiguous specimens. Several experts could not distinguish between closely related phlebotomine fly species. After realizing that multiple specimens could not be clearly and reliably identified, a barcoding analysis was executed.

A phylogenetic tree was constructed using the *cyt b* gene. This is the first phylogeny built examining the lineages of phlebotomine flies found in the southern United States. The species that had been clearly identified during morphological identification also separated clearly in the molecular analysis. The species that were ambiguous and difficult to identify

morphologically clustered in two lineages. One clade (*Micropygomyia spp.*) had four separate morphologically identified species (and one likely misidentified specimen) occupying it. The other clade (*Micropygomyia vexator*) had 6 specimens identified as one of the species in the previously mentioned *Micropygomyia spp.* clade. The *Micropygomyia spp.* clade had no genetic diversity within the *cyt b* gene and resulted in a polytomy. Interestingly enough, this lineage may represent a single species that has been erroneously split by taxonomists. Molecular techniques tend to result in splitting of species but this analysis suggests the opposite, that these different species may actually represent one species. However, more genes need to be sequenced to confirm a new taxonomic organization. The other *Micropygomyia vexator* clade had three specimens identified as a subspecies: *M. vexator occidentis.* This lineage strongly separated from the other specimens in the *Micropygomyia spp.* lineage that had been morphologically identified as *M. vexator* suggesting yet another new species.

After the morphological and molecular identifications were finished, the occurrence coordinates of the phlebotomine flies were mapped. A broad sampling of phlebotomine vectors had not been carried out before across Texas and not in areas of leishmaniasis expansion. Sampling gaps in northern, central, and eastern Texas were addressed. Several species (*Dampfomyia anthophora, Lutzomyia diabolica, Micropygomyia vexator, Psathromyia shannoni, Ps. texana*) that met three or more WHO vector criteria were found in areas of leishmaniasis emergence. Various *Micropygomyia spp.* ranges were expanded by this sampling. Additionally, *M. apache* was documented in Texas for the first time.

After identification and mapping, screening for *Le. mexicana* took place. Phlebotomine flies were pooled by site and date and tested. Rodents caught at a positive site were also tested. An extensive sensitivity analysis was performed on early stage infected flies and late state infected flies, as well as on the cloned sequence of the kinetoplast DNA minicircles. Approximately 10 copies of the kDNA minicircle sequence were able to be detected making this an extremely powerful screening method as each parasite may have up to 10,000 minicircles.

49 pools comprising 120 phlebotomine flies were tested using the kDNA minicircle primer. One phlebotomine, *D. anthophora,* located in Brazos county was positive for *Le. mexicana*. The two rodents tested at the site, *Neotoma floridana* and *Permoyscus leucopus,* were negative. This site is particularly interesting as *Ps. shannoni* (potential vector of cutaneous leishmaniasis that meets four out of five WHO criteria) as well as a potential reservoir, *N. floridana,* co-occur there. This site should be monitored for possible species jumps into *Ps. shannoni* and infection in *N. floridana.*

While this project started out with a narrow focus examining the effects of climate change on cutaneous leishmaniasis, it became more and more apparent that there were major gaps in knowledge of the location of the disease and its carriers. This realization led to the expansion of the project into field studies where vectors and reservoirs were trapped and identified. Ambiguous identifications led to molecular analyses of potential vectors which suggested that revisions to phlebotomine taxonomy be considered. And finally, after accurate categorization and identification of those phlebotomines, they were tested for *Le. mexicana.*

Ultimately, multiple research contributions were made because of this study. New occurrence data was collected that can be used for species distribution modeling of the parasite, vectors, and reservoirs. Models from this study can then be used to target specific areas of high risk for sampling. It becomes clear that while the sampling of this study was broad, the parasite was found at only one location suggesting that modeling may be more important in regards to disease risk management. Vector sampling, however, was far more successful than rodent sampling and gives us a sense of broader ecological risk.

Multiple studies are needed to examine the risk of vector-borne diseases due to climate change across taxa and the world. This study contributes by discovering that climate change has played a role in the expansion of the cutaneous leishmaniasis.

Bibliography

li-Akbarpour, M., Mohammadbeigi, A., Tabatabaee, S.H.R., and Hatam, G. (2012). Spatial Analysis of Eco-environmental Risk Factors of Cutaneous Leishmaniasis in Southern Iran. J. Cutan. Aesthetic Surg. *5*, 30–35.

Alsuhaibani, S. (1990). Field and laboratory studies of sand flies in Larimer County, Colorado. Colorado State University.

Ashford, R.W. (1996). Leishmaniasis reservoirs and their significance in control. Clin. Dermatol. *14*, 523–532.

Ashford, R.W. (2000). The leishmaniases as emerging and reemerging zoonoses. Int. J. Parasitol. *30*, 1269–1281.

Barnes, J.C., Stanley, O., and Craig, T.M. (1993). Diffuse cutaneous leishmaniasis in a cat. J. Am. Vet. Med. Assoc. *202*, 416–418.

Barve, N., Barve, V., Jiménez-Valverde, A., Lira-Noriega, A., Maher, S.P., Peterson, A.T., Soberón, J., and Villalobos, F. (2011). The crucial role of the accessible area in ecological niche modeling and species distribution modeling. Ecol. Model. *222*, 1810–1819.

Bates, P.A. (2007). Transmission of Leishmania metacyclic promastigotes by phlebotomine sand flies. Int. J. Parasitol. *37*, 1097–1106.

Bensoussan, E., Nasereddin, A., Jonas, F., Schnur, L.F., and Jaffe, C.L. (2006). Comparison of PCR Assays for Diagnosis of Cutaneous Leishmaniasis. J. Clin. Microbiol. *44*, 1435–1439.

Berzunza-Cruz, M., Rodríguez-Moreno, Á., Gutiérrez-Granados, G., González-Salazar, C., Stephens, C.R., Hidalgo-Mihart, M., Marina, C.F., Rebollar-Téllez, E.A., Bailón-Martínez, D., Balcells, C.D., et al. (2015). Leishmania (L.) mexicana Infected Bats in Mexico: Novel Potential Reservoirs. PLoS Negl Trop Dis *9*, e0003438.

Cameron, G., and Spencer, S. (1981). Sigmodon hispidus. Mammal Species *158*, 1–9.

Canto-Lara, S.B., Wynsberghe, V., R, N., Vargas-González, A., Ojeda-Farfán, F.F., and Andrade-Narváez, F.J. (1999). Use of Monoclonal Antibodies for the Identification of Leishmania spp. Isolated from Humans and Wild Rodents in the State of Campeche, Mexico. Mem. Inst. Oswaldo Cruz *94*, 305–309.

Cardenas, R., Sandoval, C.M., Rodríguez-Morales, A.J., and Franco-Paredes, C. (2006). Impact of Climate Variability in the Occurrence of Leishmaniasis in Northeastern Colombia. Am. J. Trop. Med. Hyg. *75*, 273–277.

Chable-Santos, J.B., Wynsberghe, N.R.V., Canto-Lara, S.B., and Andrade-Narvaez, F.J. (1995). Isolation of Leishmania (L.) mexicana from Wild Rodents and their Possible Role in the Transmission of Localized Cutaneous Leishmaniasis in the State of Campeche, Mexico. Am. J. Trop. Med. Hyg. *53*, 141–145.

Chaves, L.F., and Pascual, M. (2006). Climate Cycles and Forecasts of Cutaneous Leishmaniasis, a Nonstationary Vector-Borne Disease. PLoS Med *3*, e295.

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., and Thomas, C.D. (2011). Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science *333*, 1024–1026.

Christensen, H.A., and de Vasquez, A.M. (1982). The tree-buttress biotope: a pathobiocenose of Leishmania braziliensis. Am. J. Trop. Med. Hyg. *31*, 243–251.

Claborn, D.M., Rowton, E.D., Lawyer, P.G., Brown, G.C., and Keep, L.W. (2009). Species Diversity and Relative Abundance of Phlebotomine Sand Flies (Diptera: Psychodidae) on Three Army Installations in the Southern United States and Susceptibility of a Domestic Sand Fly to Infection With Old World Leishmania major. Mil. Med. *174*, 1203–1208.

Clarke, C. (2006). Emergence of Autochthonous Cutaneous Leishmaniasis in Oklahoma and Northeast Texas. In Oklahoma State Department of Health - 2006 Annual Summary, pp. 115–116.

Clarke, C.F., Bradley, K.K., Wright, J.H., and Glowicz, J. (2013). Emergence of Autochthonous Cutaneous Leishmaniasis in Northeastern Texas and Southeastern Oklahoma. Am. J. Trop. Med. Hyg. *88*, 157–161.

Craig, T.M., Barton, C.L., Mercer, S.H., Droleskey, B.E., and Jones, L.P. (1986). Dermal Leishmaniasis in a Texas Cat. Am. J. Trop. Med. Hyg. *35*, 1100–1102.

Cross, E.R., and Hyams, K.C. (1996). The potential effect of global warming on the geographic and seasonal distribution of Phlebotomus papatasi in southwest Asia. Environ. Health Perspect. *104*, 724–727.

Depaquit, J. (2014). Molecular systematics applied to Phlebotomine sandflies: review and perspectives. Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis. *28*, 744–756.

Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. *27*, 305–318.

Disney, R.H.L. (1968). Observations on a Zoonosis: Leishmaniasis in British Honduras. J. Appl. Ecol. *5*, 1–59.

Dunnum, J.L., Frey, J.K., Tinnin, D.S., Salazar-Bravo, J., and Yates, T.L. (2002). Elevational Range Extension for the Hispid Cotton Rat, Sigmodon hispidus, (Rodentia: Muridae). Southwest. Nat. *47*, 637–639.

Eads, R.B. (1978). Sandfly distribution in the United States, with a first record for Colorado (Diptera: Psychodidae) [Lutzomyia oppidana]. Proc. Entomol. Soc. Wash.

Edwards, C.W., and Bradley, R.D. (2002). Molecular systematics of the genus Neotoma. Mol. Phylogenet. Evol. *25*, 489–500.

Edwards, C.W., Fulhorst, C.F., and Bradley, R.D. (2001). Molecular phylogenetics of the neotoma albigula species group: further evidence of a paraphyletic assemblage. J. Mammal. *82*, 267–279.

Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., et al. (2006). Novel methods improve prediction of species' distributions from occurrence data. Ecography *29*, 129–151.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Yung En, C., and Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists. Divers. Distrib. *17*, 43–57.

Elnaiem, D.-E.A., Schorscher, J., Bendall, A., Obsomer, V., Osman, M.E., Mekkawi, A.M., Connor, S.J., Ashford, R.W., and Thomson, M.C. (2003). Risk Mapping of Visceral Leishmaniasis: The Role of Local Variation in Rainfall and Altitude on the Presence and Incidence of Kala-Azar in Eastern Sudan. Am. J. Trop. Med. Hyg. *68*, 10–17.

Environmental Protection Agency (2016). What climate change means for Texas.

Franco, A.O., Davies, C.R., Mylne, A., Dedet, J.-P., Gállego, M., Ballart, C., Gramiccia, M., Gradoni, L., Molina, R., Gálvez, R., et al. (2011). Predicting the distribution of canine leishmaniasis in western Europe based on environmental variables. Parasitology *138*, 1878–1891.

Franke, C.R., Ziller, M., Staubach, C., and Latif, M. (2002). Impact of El Niño/Southern Oscillation on Visceral Leishmaniasis, Brazil. Emerg. Infect. Dis. *8*, 914–917.

Freed, L.A., Cann, R.L., Goff, M.L., Kuntz, W.A., and Bodner, G.R. (2005). Increase in avian malaria at upper elevation in hawai'i. The Condor *107*, 753–764.

Fumer, B.B. (1990). Cutaneous leishmaniasis in Texas: Report of a case and review of the literature. J. Am. Acad. Dermatol. *23*, 368–371.

Genchi, C., Mortarino, M., Rinaldi, L., Cringoli, G., Traldi, G., and Genchi, M. (2011). Changing climate and changing vector-borne disease distribution: The example of Dirofilaria in Europe. Vet. Parasitol. *176*, 295–299.

Golino, A., Duncan, J., Zeluff, B., DePriest, J., McAllister, H., Radovancevic, B., and Frazier, O. (1991). Leishmaniasis in a heart transplant patient. J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant. *11*, 820–823.

Gonzales, C., Wang, O., Strutz, S.E., Gonzales-Salazar, C., Sanchez-Cordero, V., and Sarkar, S. (2010). Climate Change and Risk of Leishmaniasis in North America: Predictions from Ecological Niche Models of Vector and Reservoir Species. PLoS Negl. Trop. Dis. *4*.

Gouy, M., Guindon, S., and Gascuel, O. (2010). SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. Mol. Biol. Evol. *27*, 221–224.

Graham, C.H., Elith, J., Hijmans, R.J., Guisan, A., Townsend Peterson, A., and Loiselle, B.A. (2008). The influence of spatial errors in species occurrence data used in distribution models. J. Appl. Ecol. *45*, 239–247.

Grogl, M., Kreutzer, R.D., McHugh, C.P., and Martin, R.K. (1991). Characterization of a Leishmania Isolate from the Rodent Host Neotoma Micropus Collected in Texas and Comparison with Human Isolates. Am. J. Trop. Med. Hyg. *45*, 714–722.

Gustafson, T., Reed, C., McGreevy, P., Pappas, M., Fox, J., and Lawyer, P. (1985). Human cutaneous leishmaniasis acquired in Texas. Am. J. Trop. Med. Hyg. *34*, 58–63.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., and Samuel, M.D. (2002). Climate Warming and Disease Risks for Terrestrial and Marine Biota. Science *296*, 2158–2162.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. (2003). Biological identifications through DNA barcodes. Proc. Biol. Sci. *270*, 313–321.

Herrero, M.V., Yamell, W.E., and Schmidtmann, E.T. (2004). Landscape associations of the sand fly, Lutzomyia (Heleocyrtomyia) apache (Diptera: Psychodidae), in the southwestern United States : a geographic information system analysis. J. Vector Ecol. *29*, 205–211.

Hickling, R., Roy, D.B., Hill, J.K., Fox, R., and Thomas, C.D. (2006). The distributions of a wide range of taxonomic groups are expanding polewards. Glob. Change Biol. *12*, 450–455.

Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics *17*, 754–755.

Itah, R., Gitelman, I., and Davis, C. (2004). A replacement for methoxyflurane (Metofane) in open-circuit anaesthesia. Lab. Anim. *38*, 280–285.

Kamhawi, S. (2006). Phlebotomine sand flies and Leishmania parasites: friends or foes? Trends Parasitol. *22*, 439–445.

Kato, H., Uezato, H., Katakura, K., Calvopiña, M., Marco, J.D., Barroso, P.A., Gomez, E.A., Mimori, T., Korenaga, M., Iwata, H., et al. (2005). Detection and Identification of Leishmania Species Within Naturally Infected Sand Flies in the Andean Areas of Ecuador by a Polymerase Chain Reaction. Am. J. Trop. Med. Hyg. *72*, 87–93.

Kerr, S.F., McHugh, C.P., and Dronen, N.O. (1995). Leishmaniasis in Texas: Prevalence and Seasonal Transmission of Leishmania mexicana in Neotoma micropus. Am. J. Trop. Med. Hyg. *53*, 73–77.

Kerr, S.F., Grant, W.E., and Dronen Jr, N.O. (1997). A simulation model of the infection cycle of Leishmania mexicana in Neotoma micropus. Ecol. Model. *98*, 187–197.

Kerr, S.F., McHugh, C.P., and Merkelz, R. (1999). Short report: a focus of Leishmania mexicana near Tucson, Arizona. Am. J. Trop. Med. Hyg. *61*, 378–379.

Killick-Kendrick, R. (1990). Phlebotomine vectors of the leishmaniases: a review. Med. Vet. Entomol. *4*, 1–24.

Killick-Kendrick, R. (1999). The biology and control of Phlebotomine sand flies. Clin. Dermatol. *17*, 279–289.

Kipp, E.J. (2015). Detecting enzootic leishmaniasis and American trypanosomiasis in stray dogs in El Paso County, Texas and the potential for autochthonous transmission to humans. M.P.H. The University of Texas at El Paso.

Kipp, E.J. (2016). Reviewing Theses.

Kipp, E.J., Mariscal, J., Armijos, R.X., Weigel, M., Waldrup, K., Kipp, E.J., Mariscal, J., Armijos, R.X., Weigel, M., and Waldrup, K. (2016). Genetic evidence of enzootic leishmaniasis in a stray canine and Texas mouse from sites in west and central Texas. Mem. Inst. Oswaldo Cruz *111*, 652–654.

Kraig, S.E., Roels, S.M., and Thies, M.L. (2010). Effectiveness of Chemical Repellents in Deterring Red Imported Fire Ants (Solenopsis invicta) from Sherman Live Traps. Southwest. Nat. *55*, 203–206.

Lawyer, P.G., and Young, D.G. (1987). Experimental Transmission of Leishmania mexicana to Hamsters by Bites of Phlebotomine Sand Flies (Diptera: Psychodidae) from the United States. J. Med. Entomol. *24*, 458–462.

Lawyer, P.G., Young, D.G., Butler, J.F., and Akin, D.E. (1987). Development of Leishmania mexicana in Lutzomyia diabolica and Lutzomyia shannoni (Diptera: Psychodidae). J. Med. Entomol. *24*, 347–355.

Maloney, D.M., Maloney, J.E., Dotson, D., Popov, V.L., and Sanchez, R.L. (2002). Cutaneous leishmaniasis: Texas case diagnosed by electron microscopy. J. Am. Acad. Dermatol. *47*, 614–616.

Mariscal, J. (2013). Potential for sylvatic mammals and stray canines in transmission of leishmaniasis and Trypanosoma cruzi in Paso del Norte border area. M.P.H. The University of Texas at El Paso.

Matamoros, M.C. (2016). Identification of Leishmania spp. and Trypanosoma cruzi in sylvatic animals in El Paso County, Texas. M.P.H. The University of Texas at El Paso.

McHugh, C.P. (2003). Leishmaniasis in Washington County, Texas. J. Am. Acad. Dermatol. *49*, 1203.

McHugh, C.P. (2010). Cutaneous leishmaniasis in Texas. J. Am. Acad. Dermatol. *62*, 508–510.

McHugh, C.P., Grogl, M., and Kerr, S.F. (1990). Isolation of Leishmania mexicana from Neotoma micropus Collected in Texas. J. Parasitol. *76*, 741–742.

McHugh, C.P., Grogl, M., and Kreutzer, R.D. (1993). Isolation of Leishmania mexicana (Kinetoplastida: Trypanosomatidae) from Lutzomyia anthophora (Diptera: Psychodidae) Collected in Texas. J. Med. Entomol. *30*, 631–633.

McHugh, C.P., Melby, P.C., and LaFon, S.G. (1996). Leishmaniasis in Texas: Epidemiology and Clinical Aspects of Human Cases. Am. J. Trop. Med. Hyg. *55*, 547–555.

McHugh, C.P., Ostrander, B.F., Raymond, R.W., and Kerr, S.F. (2001). Population Dynamics of Sand Flies (Diptera: Psychodidae) at Two Foci of Leishmaniasis in Texas. J. Med. Entomol. *38*, 268–277.

McHugh, C.P., Thies, M.L., Melby, P.C., Yantis, L.D., Raymond, R.W., Villegas, M.D., and Kerr, S.F. (2003). Short report: a disseminated infection of Leishmania mexicana in an eastern woodrat, Neotoma floridana, collected in Texas. Am. J. Trop. Med. Hyg. *69*, 470–472.

Mead, D.G., and Cupp, E.W. (1995). Occurrence of Lutzomyia anthophora (Diptera: Psychodidae) in Arizona. J. Med. Entomol. *32*, 747–748.

Merkelz, R., and Kerr, S.F. (2002). Demographics, Den Use, Movements, and Absence of Leishmania mexicana in Southern Plains Woodrats (Neotoma micropus). Southwest. Nat. *47*, 70–77.

Mills, J., Gage, K., and Khan, A. (2010). Potential influence of climate change on vector-borne and zoonotic diseases: a review and proposed research plan. Environ. Health Perspect. *118*, 1507–1517.

Minter, L., Kovacic, B., Claborn, D.M., Lawyer, P., Florin, D., and Brown, G.C. (2009). New State Records for Lutzomyia shannoni and Lutzomyia vexator. J. Med. Entomol. *46*, 965–968.

Moo-Llanes, D., Ibarra-Cerdeña, C.N., Rebollar-Téllez, E.A., Ibáñez-Bernal, S., González, C., and Ramsey, J.M. (2013). Current and Future Niche of North and Central American Sand Flies (Diptera: Psychodidae) in Climate Change Scenarios. PLoS Negl Trop Dis *7*, e2421.

Nakazawa, Y., Williams, R., Peterson, A.T., Mead, P., Staples, E., and Gage, K.L. (2007). Climate Change Effects on Plague and Tularemia in the United States. Vector-Borne Zoonotic Dis. *7*, 529–540.

Nelson, D.A., Gustafson, T.L., and Spielvogel, R.L. (1985). Clinical aspects of cutaneous leishmaniasis acquired in Texas. J. Am. Acad. Dermatol. *12*, 985–992.

Ostfeld, R.S. (2009). Climate change and the distribution and intensity of infectious diseases. Ecology *90*, 903–905.

Parker, W.T., Muller, L.I., Gerhardt, R.R., O'rourke, D.P., and Ramsay, E.C. (2008). Field Use of Isoflurane for Safe Squirrel and Woodrat Anesthesia. J. Wildl. Manag. *72*, 1262–1266.

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. Annu Rev Ecol Evol Syst *37*, 637–669.

Parmesan, C., and Martens, L.A. (2009). Climate change, wildlife, and human health. In Biodiversity Change and Human Health: From Ecosystem Services to Spread of Disease, (Island Press), pp. 245–266.

Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. Nature *421*, 37–42.

Patz, J.A., and Olson, S.H. (2006). Climate change and health: global to local influences on disease risk. Ann. Trop. Med. Parasitol. *100*, 535–549.

Pearson, R.G., Raxworthy, C.J., Nakamura, M., and Townsend Peterson, A. (2007). Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. J. Biogeogr. *34*, 102–117.

Pech-May, A., Escobedo-Ortegon, F.J., Berzunza-Cruz, M., and Rebollar-Téllez, E.A. (2010). Incrimination of four sandfly species previously unrecognized as vectors of Leishmania parasites in Mexico. Med. Vet. Entomol. *24*, 150–161.

Perkins, P.V., Endris, R.G., and Young, D.G. (1987a). Experimental transmission of Leishmania mexicana by a North American sand fly, Lutzomyia anthophora (Diptera: Psychodidae). J. Med. Entomol. *24*, 243–247.

Perkins, P.V., Endris, R.G., and Young, D.G. (1987b). Experimental transmission of Leishmania mexicana by a North American sand fly, Lutzomyia anthophora (Diptera: Psychodidae). J. Med. Entomol. *24*, 243–247.

Perry, A.L., Low, P.J., Ellis, J.R., and Reynolds, J.D. (2005). Climate Change and Distribution Shifts in Marine Fishes. Science *308*, 1912–1915.

Phillips, S.J., and Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography *31*, 161–175.

Phillips, S.J., Dudik, M., and Schapire, R.E. (2017a). Maxent software for modeling species niches and distributions.

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., and Blair, M.E. (2017b). Opening the black box: an open-source release of Maxent. Ecography *40*, 887–893.

Polley, L., and Thompson, R.C.A. (2009). Parasite zoonoses and climate change: molecular tools for tracking shifting boundaries. Trends Parasitol. *25*, 285–291.

Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P.J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T., et al. (2013). Global imprint of climate change on marine life. Nat. Clim. Change *3*, 919–925.

Raun, G.G. (1966). A population of woodrats (Neotoma micropus) in southern Texas (Texas Memorial Museum).

Raymond, R.W., McHugh, C.P., Witt, L.R., and Kerr, S.F. (2003). Temporal and spatial distribution of Leishmania mexicana infections in a population of Neotoma micropus. Mem Inst Oswaldo Cruz *98*, 171–180.

R Core Team (2013). R: A language and environment for statistical computing. R Found. Stat. Comput.

Ready, P.D. (2013). Biology of Phlebotomine Sand Flies as Vectors of Disease Agents. Annu. Rev. Entomol. *58*, 227–250.

Reed, C. (1986). Autochthonous human cutaneous leishmaniasis in Texas. Epidemiol Bull *13*, 1–5.

Reeves, W.K., Kato, C.Y., and Gilchriest, T. (2008). Pathogen Screening and Bionomics of Lutzomyia apache (Diptera: Psychodidae) in Wyoming, USA. J. Am. Mosq. Control Assoc. *24*, 444–447.

Reithinger, R., Dujardin, J.-C., Louzir, H., Pirmez, C., Alexander, B., and Brooker, S. (2007). Cutaneous leishmaniasis. Lancet Infect. Dis. *7*, 581–596.

Rogers, W.O., and Wirth, D.F. (1987). Kinetoplast DNA minicircles: regions of extensive sequence divergence. Proc. Natl. Acad. Sci. *84*, 565–569.

Rosenthal, S.R., Ostfeld, R.S., McGarvey, S.T., Lurie, M.N., and Smith, K.F. (2015). Redefining disease emergence to improve prioritization and macro-ecological analyses. One Health *1*, 17–23.

Rougeron, V., De Meeûs, T., Hide, M., Waleckx, E., Bermudez, H., Arevalo, J., Llanos-Cuentas, A., Dujardin, J.-C., De Doncker, S., Le Ray, D., et al. (2009). Extreme inbreeding in Leishmania braziliensis. Proc. Natl. Acad. Sci. *106*, 10224–10229.

Rougeron, V., De Meeûs, T., Kako Ouraga, S., Hide, M., and Bañuls, A.-L. (2010). "Everything You Always Wanted to Know about Sex (but Were Afraid to Ask)" in Leishmania after Two Decades of Laboratory and Field Analyses. PLoS Pathog *6*, e1001004.

Rougeron, V., Banuls, A.-L., Carme, B., Simon, S., Couppie, P., Nacher, M., Hide, M., and De Meeus, T. (2011). Reproductive strategies and population structure in Leishmania: substantial amount of sex in Leishmania Viannia guyanensis. Mol. Ecol. *20*, 3116–3127.

Schmidly, D.J. (2002). Texas Natural History: A Century of Change (Texas Tech University Press).

Schmidly, D.J. (2004). The Mammals of Texas: Revised Edition (University of Texas Press).

Schmidtmann, E.T., Craig, M.E., English, L.M., and Herrero, M.V. (2002). Sampling for Sand Flies (Diptera: Psychodidae) Among Prairie Dog Colonies on Ranches with Histories of Vesicular Stomatitis in New Mexico and Colorado. J. Med. Entomol. *39*, 680–684.

Schönian, G., Mauricio, I., and Cupolillo, E. (2010). Is it time to revise the nomenclature of Leishmania? Trends Parasitol. *26*, 466–469.

Shaw, P., Quigg, L., Allain, D., Juranek, D., and Healy, G. (1976). Autochthonous dermal leishmaniasis in Texas. Am. J. Trop. Med. Hyg. *25*, 788–796.

Shimabukuro, P.H.F., Andrade, A.J. de, and Galati, E.A.B. (2017). Checklist of American sand flies (Diptera, Psychodidae, Phlebotominae): genera, species, and their distribution. ZooKeys *660*, 67–106.

Shlomai, J. (2004). The structure and replication of kinetoplast DNA. Curr. Mol. Med. *4*, 623–647.

Simpson MH, Mullins J, and Stone OJ (1968). Disseminated anergic cutaneous leishmaniasis: An autochthonous case in texas and the mexican states of tamaulipas and nuevo leon. Arch. Dermatol. *97*, 301–303.

Stewart, C., and Pilcher, J. (1945). American leishmaniasis: Report of an autochthonous case. Arch. Dermatol. Syphilol. *51*, 124–128.

Thompson, R.A., Lima, J.W. de O., Maguire, J.H., Braud, D.H., and Scholl, D.T. (2002). Climatic and demographic determinants of American visceral leishmaniasis in northeastern Brazil using remote sensing technology for environmental categorization of rain and region influences on leishmaniasis. Am. J. Trop. Med. Hyg. *67*, 648–655.

Thomson, M.C., Elnaiern, D.A., Ashford, R.W., and Connor, S.J. (1999). Towards a kala azar risk map for Sudan: mapping the potential distribution of Phlebotomus orientalis using digital data of environmental variables. Trop. Med. Int. Health *4*, 105–113.

Toumi, A., Chlif, S., Bettaieb, J., Alaya, N.B., Boukthir, A., Ahmadi, Z.E., and Salah, A.B. (2012). Temporal Dynamics and Impact of Climate Factors on the Incidence of Zoonotic Cutaneous Leishmaniasis in Central Tunisia. PLoS Negl Trop Dis *6*, e1633.

Trainor, K.E., Porter, B.F., Logan, K.S., Hoffman, R.J., and Snowden, K.F. (2010). Eight Cases of Feline Cutaneous Leishmaniasis in Texas. Vet. Pathol. Online *47*, 1076–1081.

Vilcins, I. (2016). Leishmaniasis in Texas, 2007-2014. (Omni San Antonio Hotel at the Colonnade, San Antonio, Texas),.

Weng, J.-L., Young, S.L., Gordon, D.M., Claborn, D., Petersen, C., and Ramalho-Ortigao, M. (2012). First report of Phlebotomine sand flies (Diptera: Psychodidae) in Kansas and Missouri, and a PCR method to distinguish Lutzomyia shannoni from Lutzomyia vexator. J. Med. Entomol. *49*, 1460.

Williams, P. (1966). Experimental transmission of Leishmania mexicana by Lutzomyia cruciata. Ann Trop Med Parasitol *60*, 365–372.

World Health Organization (2004). The world health report 2004 - changing history.

World Health Organization (2010). Control of the leishmaniases:Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases (Geneva, Switzerland: World Health Organization).

Wright, N.A., Davis, L.E., Aftergut, K.S., Parrish, C.A., and Cockerell, C.J. (2008). Cutaneous leishmaniasis in Texas: A northern spread of endemic areas. J. Am. Acad. Dermatol. *58*, 650–652.

Van Wynsberghe, N.R., Canto-Lara, S.B., Sosa-Bibiano, E.I., Rivero-Cárdenas, N.A., and Andrade-Narváez, F.J. (2009). Comparison of small mammal prevalence of Leishmania (Leishmania) mexicana in five foci of cutaneous leishmaniasis in the State of Campeche, Mexico. Rev. Inst. Med. Trop. São Paulo *51*, 87–94.

Young, D.G. (1972). Phlebotomine Sand Flies from Texas and Florida (Diptera: Psychodidae). Fla. Entomol. *55*, 61–64.

Young, D.G., and Perkins, P.V. (1984). Phlebotomine sand flies of North America (Diptera: Psychodidae) [Lutzomyia]. Mosq. News USA.

Zeledón, R., and Alfaro, M. (1973). Isolation of Leishmania braziliensis from a Costa Rican sandfly and its possible use as a human vaccine. Trans. R. Soc. Trop. Med. Hyg. *67*, 416–417.