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Disease Surveillance and Projected Expansion in Climatic Suitability for *Trypanosoma cruzi*, the Etiological Agent of Chagas Disease, in Oklahoma

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Disease Surveillance and Projected Expansion in Climatic Suitability for *Trypanosoma cruzi*, the Etiological Agent of Chagas Disease, in Oklahoma

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ABSTRACT OF THESIS

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TITLE OF THESIS: Disease Surveillance and Projected Expansion in Climatic Suitability for *Trypanosoma cruzi*, the Etiological Agent of Chagas Disease, in Oklahoma

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ABSTRACT: The vector-borne hemoflagellate parasite *Trypanosoma cruzi* infects seven million people globally and causes chronic cardiomyopathy and gastrointestinal diseases. Historically, *T. cruzi* was endemic to Central and South America, but is now found throughout the southern United States and across 43 countries globally. There are three reports of *T. cruzi* in wild raccoons and dogs in Oklahoma, but its endemicity in the state is poorly studied. We suspect Mexican free-tailed bats (*Tadarida brasiliensis*) contribute to the endemicity of *T. cruzi* in Oklahoma by their annual migration from Central America to North American maternity roosts. During the summer of 2017, we sampled 361 Mexican free-tailed bats at three maternity roosts in Oklahoma for *T. cruzi*. We collected wing tissues and extracted DNA, amplified target *T. cruzi* DNA by PCR using the primers TCZ1/TCZ2, and observed amplification by gel electrophoresis. We detected *T. cruzi* DNA in one juvenile Mexican free-tailed bat resulting in a prevalence of 0.27% in the 361 sampled bats. The positive sample was sequenced at Eton Biosciences,

confirmed as *T. cruzi*, and uploaded to GenBank (MG869732). This finding is the first reported detection of a wild bat naturally infected with *T. cruzi* in Oklahoma, suggests Mexican free-tailed bats can contribute to *T. cruzi* endemicity via migration between endemic foci, and provides insight on the endemicity of *T. cruzi* in underrepresented endemic areas. To better understand the potential impact of global climate change on the future epidemiology of *T. cruzi* in Oklahoma, we used the program MaxEnt to develop an ecological niche model for *T. cruzi* and five widespread *Triatoma* vectors based on 19 bioclimatic variables and 546 published localities within the United States. We modeled regions of current potential *T. cruzi* and *Triatoma* distribution, and regions projected to have suitable climatic conditions under a Representative Concentration Pathway (RCP 8.5) scenario by 2070. Regions with potential suitable climatic conditions for *T. cruzi*, *T. indictiva, T. lecticularia, T. protracta*, and *T. sanguisuga* are predicted to increase within the United States and Oklahoma by 2070. Regions with potential suitable climatic conditions for *T. gerstaeckeri* are predicted to increase within the United States but not in Oklahoma by 2070. Our findings agree with previous literature and confirm that climate change will influence the expansion of *T. cruzi* and important *Triatoma* vectors in Oklahoma and the United States.

GLOSSARY

Term	Definition
Accidental host	The parasite infects a host that is not readily infected by the parasite
Accidental parasite	The parasite may be ingested by a host species that is different from its preferred host
Amastigote	The aflagelled form that contains a centrally located kinetoplast, no undulating membrane, and found in cells and tissues of infected vertebrates
Commensalism	An interaction in which one organism increases its fitness and the other organism neither benefits nor is harmed
Dead end host	The parasite cannot be transmitted to another host or vector
Definitive host	The parasite reaches sexual maturity in this host
Ectoparasite	A parasite that lives on the surface of its hosts
Endoparasite	A parasite that lives within the body of its hosts
Epimastigote	The flagellated form that contains a centrally located kinetoplast, undulating membrane, and found in the gut of infected invertebrates
Epiparasite	A parasite that feeds on other parasites
Etiological agent	The causative organism of a disease or infection
Facultative parasite	Not parasitic in the wild but can become so when inside a host
Helminth	A multicellular worm
Hemoflagellate	An organism that lives in the blood and contains a flagellum
Host	An organism infected by a parasite and nourishes or supports a parasite through a developmental stage
Intermediate host	The parasite develops but does not reach sexual maturity
Kinetoplast	An organelle that regulates genes and allows the trypanosome to switch between infected invertebrate and vertebrate hosts
Macroparasite	A parasitic organism that can be seen with the naked eye

Mesoparasite	The parasite lives in an intermediate position, between endoparasitism and ectoparasitism
Metacyclic trypomastigote	The flagellated form that resembles a trypomastigote but is found in the rectum of infected invertebrates
Microparasite	A parasitic organism that is too small to see without a microscope
Mutualism	An interaction in which two or more organisms increase their overall fitness through communal interactions
Obligate parasite	The parasite cannot complete its life cycle without parasitizing a host
Parasite	An animal or plant living in or on another organism and getting its nutrients from it
Parasitism	An interaction in which one participant, the parasite, either harms its host or develops and persists at the expense of the host
Parasitology	The scientific study of parasites
Paratenic host	The parasite requires a host to complete the life cycle but does not develop
Permanent parasite	The parasite lives its entire adult life within or on its host
Phoresis	An interaction in which an organism is physically relocated by another organism
Predilection host	The host that is most preferred by the parasite
Protozoan	A unicellular organism with a membrane bound nucleus
Reservoir	An animal or species that is readily infected by a parasite and serves as a sentinel or source of infection to other hosts
Social parasite	The parasite parasitizes a large group of organisms to get its nutrient supply
Symbiosis	The scientific study of the interactions between different organisms
Temporary parasite	The parasite feeds on its host and then leaves after obtaining its nutrient supply
Transport	A host is exploited by a parasite until the appropriate or preferred host is reached but is not required to complete the life cycle

Triatomine	A hematophagous insect within the subfamily Triatominae; capable of transmitting *Trypanosoma cruzi*
Trypanosome	A member of the Trypanosomatidae family; these protozoan organisms cause infectious diseases within their hosts
Trypomastigote	The flagellated form that contains a terminally located kinetoplast, undulating membrane, and found in the blood of infected vertebrates
Undulating membrane	A large structure that aids in locomotion
Vector	Living organisms that transmit pathogens to others
Vector-borne parasite	A parasitic organism that is transmitted by a vector; usually a hematophagous arthropod

THESIS INTRODUCTION

Parasitology and Parasitism

Parasitology is defined as a study of symbiosis and the scientific study of parasites (Larry and Gerald 2000). The word "symbiosis" is derived from Ancient Greek meaning "living together" and is observed by close and long-term interactions between two or more biological species (Liddel et al. 1940). Due to the wide diversity of biological interactions, four different types of symbiotic relationships have emerged: 1) parasitism, 2) mutualism, 3) commensalism, 4) phoresis (Douglas 2010; Martin and Schwab 2012). Parasitism is a relationship in which one of the participants, typically the parasite, either harms its host or develops and persists at the expense of the host (Larry and Gerald 2000). Mutualism is an interaction in which both organisms increase their fitness through their communal interactions. Commensalism is an interaction in which one organism increases its fitness and the other organism neither benefits nor is harmed. Phoresis is an interaction in which an organism, the phoront, is physically relocated by an organism.

A parasite is defined as an animal or plant living in or on another organism and getting its food from it. The word "parasite" is derived from the Latin word "parasitus", which means one who eats at the table of another (Liddel et al. 1940). Traditionally, parasites were a group of organisms visible to the naked eye (macro-parasites such as helminths), but currently parasites now include microscopic organisms such as protozoans, viruses, and bacteria, which are called micro-parasites (Combes 2005).

In parasitic interactions, a host organism is infected with or fed upon by a parasitic organism. A host is an organism that nourishes and supports a parasite through at least one developmental stage (Larry and Gerald 2000). The host does not benefit from the parasitic

relationship but may be harmed by the association. Despite the potential harm a parasite may elicit on its host, it is not often beneficial for the parasite to kill its host. Parasites vary in host competency and specificity (Solomon et al. 2015). A parasite species can infect one or more hosts, although most parasites occur on a restricted number of hosts (Solomon et al. 2015). Parasite host specificity is defined based on the number of hosts a parasite is capable of infecting at a given time (Solomon et al. 2015). Host specific parasites often have a definitive host, but may have a limited number of less frequently parasitized hosts in the absence of the definitive host (Poulin 1992; Tripet et al. 2002). Among less specific parasites, there is still a preference for certain hosts (Tripet and Richner 1997).

There are eight types of hosts (Jaenike 1990; Thompson 1994; Combes 1997; Norton and De Lange 1999; McCoy et al. 2001; Solomon et al 2015). The first type of host is a definitive or primary host, which represents an organism in which a parasite reaches sexual maturity. The second type of host is an intermediate host, which is an organism in which a parasite develops but does not reach sexual maturity. The third type is a paratenic host, in which a host is required for the completion of a parasite's life cycle, but the parasite does not develop while in or on the paratenic host. The fourth type is an accidental host, which occurs when an organism accidentally harbors a parasitic organism that does not readily parasitize that host species. The fifth type is a dead-end host, in which the disease cannot be transmitted to another animal, human, or vector. The sixth type is a predilection host, which is the host that is most preferred by the parasite. The seventh type is a reservoir host, which is an animal or species that is readily infected by a parasite and serves as a sentinel or source of infection for humans or other species. The eighth type of host is a transport host, which is a host exploited by a parasite until the

appropriate or preferred host is reached but is not required to complete the life cycle of the parasite.

There are ten current types of parasites (Solomon et al. 2015). The first are ectoparasites, such as ticks, which live on the surface of their hosts. The second are endoparasites, such as intestinal amoeba, which live within the body of their hosts. The third are obligate parasites, such as *Toxoplasma gondii* which cannot complete their life cycle without spending at least part of their development in a parasitic relationship. The fourth are facultative parasites, such as *Naegleria fowleri*, which are not often parasitic in the wild but can become so when accidentally inhaled by a host while swimming. The fifth type are accidental parasites, such as *Baylisascaris procyonis,* which the parasite may be ingested by a host species that is different from its preferred host, the raccoon. The sixth are permanent parasites, such as mature schistosome helminths, which are parasites that live their entire adult lives within their hosts. The seventh are temporary parasites, such as bed bugs (*Cimex* spp.) which feed on their hosts and then leave once their nutrient supply is obtained. The eighth type of parasites are mesoparasites, which are parasites living in an intermediate position, often partially ectoparasitic and partially endoparasitic, such as *Kroyeria caseyi*, a parasitic copepod which lives in the gills of night sharks (Benz and Deets 1986). The ninth type of parasites are epiparasites, which feed on other parasites, such as the mycorrhizal fungi which feeds on the roots of some parasitic plants. The tenth type of parasites are social parasites, such as *Polyergus breviceps,* which parasitize other groups of ants and employ them for nestmaking.

Natural selection tends to shape the specialization of parasites to their local environment and hosts (Combes 1991; Thompson 1994; Kawecki 1998). Parasites face fierce competition within their ecosystem and better adapted and more fit hosts and parasites exist in greater

abundance than lesser adapted and less fit hosts and parasites. Host specialization is promoted by host dependent fitness, availability, and predictability (De Meus et al. 1998; Jaenike 1990). A parasite may specialize if it is advantageous to the longevity of the parasite, thus having a single host species may outweigh the benefits of parasitizing multiple species (Jaenike 1990). The lack of adequate hosts will promote parasite generalization, while the abundance of different hosts will promote parasite specialization (Tripet and Richner 1997; Soler et al. 1999; Tripet et al. 2002).

Evolution of Parasitism

Biotrophic parasitism is widespread among different ecosystems and rose independently through the course of evolution (Combes 2005; Solomon et al. 2015). Nearly half of all living animals have one or more parasitic phases in their life cycles and almost all free-living animals are hosts to one or more parasites at one time (Price 1980). The success of modern day parasites is attributed to their evolutionary response to their host's defense mechanisms. Because of the host defenses, some parasites evolved specific adaptations to a host taxon, specializing so much that the parasite may only parasitize a limited number of species (Solomon et al. 2015). Alternatively, host defenses evolved in response to parasitic infections. Several successful parasites coevolved with their host taxa and led to a long-term relationship that slightly resembles a commensalistic relationship, because it is in the parasite's best interest to keep the host alive (Solomon et al. 2015). Furthermore, a parasite might evolve to become less pathogenic towards its host or a host might evolve a new coping mechanism to persist with the inevitable infection and this has led to numerous host species that harbor certain parasites while nearly unharmed, such as the relationship between the domesticated cow, *Bos taurus* and the beef tapeworm, *Taenia saginata* (Solomon et al. 2015).

Parasitic infections may modify the behavior of an infected host to favor transmission. This is observed in the widespread protozoan parasite *Toxoplasma gondii*, which naturally matures in its definitive host, the cat. *Toxoplasma gondii* is capable of infecting nearly every mammal. However, it is highly prevalent in rodent species, in which the parasite can manipulate the behavior of the infected rodents. Uninfected rodents typically avoid cat odors, whereas infected rodents are attracted to cat odors and even show combative behavior towards cats, which causes the rodent to be easily devoured and transmission potential is increased (Berdoy et al. 2000).

Perhaps the success of parasites is a byproduct of their involvement within food webs, as they can occupy the top trophic position (Solomon et al. 2015). Parasites can function as a keystone species within their ecosystem by reducing the abundance of dominant competitors and promote competing species to cohabitate. Additionally, numerous parasites require multiple hosts from different species to complete their life cycles and this relies on both predator to prey involvements as well as other ecological interactions to pass from one host to another. Parasites function as an ecosystem equilibrator and account for nearly half of life's diversity (Solomon et al. 2015).

Parasite Categories

There are two broad categories of parasites, the protozoa and the helminths. Parasitic protozoa and helminths both evolved survival mechanisms that divert or inhibit the host's immune response, avoid or inhibit intracellular killing mechanisms, and infect areas of the host where there is low immune surveillance and responses (Despommier et al. 2017). A protozoan is one of over 200,000 named species of unicellular organisms with typically one membrane bound nucleus, although some exceptions include *Giardia lamblia* and *Dientamoeba fragilis*

(Despommier et al. 2017). Most protozoa contain one structure that aids in locomotion, such as a flagellum, undulating membrane, or cilia. Free living protozoa are found in every ecological niche, from marine trenches to glaciers. For parasitic protozoa, the host often provides the required nutrients for metabolic processes (Despommier et al. 2017), which vary between anaerobic and aerobic pathways. Parasitic protozoa often employ remarkable reproductive strategies within a host. Parasitic protozoa can replicate within any given host and produce hundreds of thousands of new parasites within days of an initial infection. Nearly all described parasitic protozoa reproduce asexually via binary fission after entering their definitive host, which directly influences host immune response and pathological consequences (Despommier et al. 2017). Protozoa are often niche parasites, thus frequently occupying one region of the host.

Helminths are grouped within four phyla: Nematoda (roundworms), Platyhelminthes (flatworms), Acanthocephala (spiny headed worms), and Nematophora (hairworms). In humans, only the nematodes and platyhelminths are endoparasitic (Despommier et al. 2017). There are multiple free-living species within the Nematoda and Platyhelminthes. Helminths are multicellular and are among the most abundant organisms worldwide (Despommier et al. 2017). Helminth infections usually are established by exposure to an environmentally resistant stage, such as an egg or larva. Helminth eggs are found in all environments and have even been recovered from paper currency (Despommier et al. 2017). Many parasitic helminths reside as sexually mature adults within the gastrointestinal tract; however, unlike parasitic protozoa, many species of parasitic helminths utilize more than one niche within a host. In several underdeveloped countries, children often harbor three or four species of helminths in their intestine, with each species occupying a different region of their gut (Despommier et al. 2017).

Helminths are specialized for mass reproductive strategies, often producing several thousand eggs per day (Despommier et al. 2017).

Parasite Transmission

Endoparasites face numerous challenges elicited by the host, such as immune responses and the ability to pass their offspring to future hosts. Parasites developed numerous strategies to evade host defenses and to ensure their safe passage from host to host (Solomon et al. 2015). The transfer between hosts is called colonization. Parasite colonization may occur through numerous pathways, such as penetrating the host skin, being inhaled or ingested in infected organic matter, sexually, or through an insect capable of penetrating the host's tissues and allowing the parasite a portal inside the host (Solomon et al. 2015). While inside the host, mature endoparasites must replicate, encyst in tissue and wait to be ingested by another host, or shed offspring to the external environment to perpetuate their life cycle in other hosts. Many endoparasites, such as helminths, live in the gastrointestinal tract and easily shed eggs along with the host's excrement. Other endoparasites, such as malaria parasites and trypanosomes, use insect vectors to transmit their infective stages to various hosts (Solomon et al. 2015). Some endoparasite larval stages infect other host regions, such as muscle tissue. These endoparasite larva rely on their host to be ingested by the next host, which they can then colonize, mature, and reproduce. Alternatively, some endoparasite larva can be shed externally and are free living until they colonize a host (Solomon et al. 2015).

Ectoparasites face different challenges than endoparasites, such as environmental factors or host scarcity. Many ectoparasites rely on direct contact between hosts and may shed eggs which survive off the host or wait within the external environment to interact with a new host (Solomon et al. 2015).

Vector-borne Parasites

Vectors are living organisms capable of transmitting pathogens that cause infectious diseases. Often vectors transmit pathogens between humans or from animals to humans (World Health Organization (WHO) 2017)). Many vectors are hematophagous arthropods, such as mosquitoes, ticks, and hemipterans, which ingest pathogenic microorganisms during a blood meal from an infected host and later inject or deposit the pathogen on a new host during the subsequent blood meal (WHO 2017 http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Mosquitoes are classified as the world's deadliest animal and are competent vectors for malaria parasites, Dengue fever, yellow fever, and other pathogens (WHO 2017 http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Vector-borne diseases account for more than 17% of all infectious diseases and cause an estimated 700,000 deaths annually (WHO 2017 http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Over 3.9 billion people are at risk of contracting Dengue fever, and malaria causes over 400,000 deaths annually, most of them in children under 5. Diseases such as Chagas disease, African trypanosomiasis, leishmaniasis, and schistosomiasis affect hundreds of millions of people globally (WHO 2017 http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Many vector-borne diseases are preventable by protective measures against the vector. The global burden of vector-borne diseases is highest in tropical and subtropical areas. Vector-borne disease transmission and morbidity are impacted by global travel, urbanization, and environmental challenges caused by global climate change which can extend the transmission season and promote tropical diseases to emerge in temperate regions (WHO 2017 http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases).

Trypanosomatidae

Protozoa within the Trypanosomatidae family are specialized hemoflagellate parasites that have a high prevalence and marked negative economic impact in developing countries (Souza et al. 2010). African sleeping sickness, caused by *Trypanosoma brucei* species affects three million individuals in Africa. Leishmaniasis, caused by multiple *Leishmania* species, affects roughly 16 million individuals in Africa, Asia, Europe, and Latin America. *Trypanosoma cruzi* causes Chagas disease, or American trypanosomiasis, in seven million individuals, most of which are in Latin America (Souza et al. 2010). A shared feature of the trypanosomatids is the ability to change their shape throughout their life cycle, depending on which host they are in. Trypanosomatids switch between vertebrate and invertebrate hosts to complete their life cycles through an evolved process called protozoan differentiation (Souza et al. 2010). Of the trypanosomatids, *T. cruzi* employs one of the most complex life cycles involving multiple developmental stages within the invertebrate vector and vertebrate host cells (Souza et al. 2010).

Trypanosoma cruzi

Trypanosoma cruzi is a vector-borne protozoan parasite and the etiological agent of American Trypanosomiasis, commonly known as Chagas disease. Approximately seven million people across 43 countries are infected with *T. cruzi,* and 25 million are at risk for infection (Bern et al. 2011; WHO 2018 http://www.who.int/chagas/en/). Of the infected individuals, up to 40% will develop clinical Chagas disease, which is the leading cause of cardiomyopathy in endemic regions (Bocchi et al. 2009; Rassi et al. 2010). Current estimates of infected persons in the United States sum to 300,000 (Centers for Diseases Control and Prevention (CDC) 2018)). Chagas disease is marked as a neglected tropical disease (Bern et al. 2009), ranked highest in parasitic disease burden estimates for the Americas (WHO 2018 http://www.who.int/chagas/en/), and accounts for roughly fivefold disability-adjusted life years lost when compared to malaria

(Bern et al. 2009; WHO 2018 http://www.who.int/chagas/en/). If untreated, infected hosts can develop chronic Chagas disease, which manifests as cardiomyopathy, tissue fibrosis, lethargy, digestive megasyndromes such as megacolon and megaesophagus, malaise, and 10,000 deaths annually (Rassi et al. 2010). *T. cruzi* has been shown to infect over 400 mammalian species (Hoare 1972) and is transmitted when an infected triatomine insect (Hemiptera: Reduviidae: Triatominae), commonly called kissing bugs, feeds on a mammalian host and defecates the parasite onto the host. The infected feces enters the bite wound or a mucous membrane (Rassi et al. 2010; Bern et al. 2011). *T. cruzi* also may be transmitted through blood transfusions, organ transplants, congenitally, and orally (Schmunis 1991; Torrico et al. 2004; Roellig et al. 2009; Chin-Hong et al. 2011; Barbosa et al. 2012; Barreto-de-Albuquerque et al. 2015). Blood donation screenings were implemented to prevent the transmission of *T. cruzi*, but before mandating the screening in 2006, the risk of transmission was 10-25% (Bern et al. 2008). Schmunis (1991) recorded *T. cruzi* prevalence in donated blood ranging from 1% to 60% in Latin American cities. Although oral transmission is underreported, transmission occurs frequently through ingestion of infected triatomine feces with contaminated food and water in endemic South American countries, causing severe acute infections with systemic dissemination and 28.6% mortality (Dias et al. 2008; Silva-dos-Santos et al. 2017). Recent outbreaks in the Brazilian Amazon report 71% of acute cases were attributed to the ingestion of contaminated food and beverages (Secretaria de Vigilancia em Saude 2015).

Historically, transmission and morbidity were concentrated in rural areas of Latin America where poor housing conditions and inadequate vector control allowed high vector infestations (Mott et al. 1978; Bern et al. 2011). Over the last few decades, *T. cruzi* infections have globalized due to human immigration from endemic areas to non-endemic areas, such as

the United States, Europe, and Asia (Bern et al. 2011). Alternatively, recent successful vector surveillance and control programs from Latin America and the World Health Organization have decreased transmission in rural areas and created an initiative to combat the globalization of Chagas disease and increase awareness for nonvectorial transmission (WHO 2007 http://apps.who.int/iris/bitstream/handle/10665/240973/WER8228_29_259-260.PDF?sequence=1).

Trypanosoma cruzi Life Cycle

Carlos Chagas first identified and described the *T. cruzi* life cycle in 1909. *T. cruzi* is a kinetoplastid protozoan (Kinetoplastida: Trypanosomatidae) which possesses an organelle that regulates genes allowing the parasite to utilize vertebrate and invertebrate hosts to complete its life cycle (Rassi et al. 2010). The life cycle (Fig. 1) begins when a triatomine ingests circulating, flagellated trypomastigotes during a blood meal from an infected mammalian host (Rassi et al. 2010). A trypomastigote is a) characterized by a large undulating membrane that attaches to the flagellum, possessing a terminally located kinetoplast and centrally located nucleus, b) found in the circulating blood of a vertebrate host, and c) characteristic of acute infection (Rassi et al. 2010). Trypomastigotes transform into epimastigotes, travel to the triatomine's midgut, and continually replicate by binary division before attaching to the perimicrovillar membranes in the intestinal cells (Gonzalez et al. 1999; Rassi et al. 2010). Epimastigotes have a thin flagellum, centrally located kinetoplast and nucleus, colonize the triatomine gut until the invertebrate dies, and are found only in the invertebrate host (Rassi et al. 2010). A portion of epimastigotes travel to the hindgut, attach to the rectal cuticle, and differentiate into infective metacyclic trypomastigotes, which are defecated onto the host after each blood meal (Garcia and Azambuja 1991; Kollien and Schaub 2000).

The metacyclogenesis of various *T. cruzi* strains are related to the biology of *T. cruzi* and its susceptibility to its colonized triatomine species (Garcia et al. 1986). Different factors influence the successful establishment of *T. cruzi* in the invertebrate gut, such as crop lytic factors, which lyse the erythrocyte membrane and release free hemoglobin for digestion, lectins which increase infectivity within the gut, and peptides released from digesting a component of the α^D globin chain, which induce metacyclogenesis (Azambuja et al. 1983; Franidenraich et al. 1993; Garcia et al. 1999). *In vitro,* the transformation from epimastigotes to metacyclic trypomastigotes inside the invertebrate gut is induced if either hemoglobin or peptides corresponding to residues 30-49 and 35-73 of the α^D globin chain are added to an infective plasma diet (Garcia et al. 1995). Garcia et al. (1995) suggest the parasite transformation is facilitated by globin fragments released by proteolytic enzymes that attack hemoglobin. The metacyclic trypomastigotes enter the bite wound through physical inoculation by the host, typically from rubbing or scratching the wound (Rassi et al. 2010; Bern et al. 2011). Metacyclic trypomastigotes stimulate a local infection at the bite site called a chagoma or when penetrating the mucous membrane of the eye, causing unilateral periorbital edema commonly called Romana's sign (Rassi et al. 2000).

Once inside the mammalian host, metacyclic trypomastigotes invade local reticuloendothelial and connective tissue cells and become aflagellated amastigotes (Rassi et al. 2000). Although all types of nucleated cells are capable of invasion, adipocytes are targets for acute infections by trypomastigotes and serve as a reservoir of amastigotes during chronic infections due to the longevity of adipocytes (half-life of 10 years), decreased immune surveillance, constant supply of intracellular nutrients such as fatty acids, and postprandial surges of insulin to maintain a steady supply of glucose (Combs et al. 2005; Spalding et al. 2008; Rassi

et al. 2010; Ferreira et al. 2011; Nagajyothi et al. 2012; Tanowitz et al. 2017). The low-density lipoprotein receptor (LDLr) is the primary target utilized by *T. cruzi* to bind and invade adipocytes and alter intracellular cholesterol homeostasis (Nagajyothi et al. 2011; Johndrow et al. 2014). Other entry mechanisms detail two main steps: adhesion and internalization (Barrias et al. 2013). These involve a variation of the endocytic pathway, such as phagocytosis, active entry, membrane microdomain dependent endocytosis, clathrin-mediated endocytosis, and micropinocytosis (Barrias et al. 2013).

Cellular adhesion and invasion begins with *T. cruzi* surface glycoproteins such as mucins (Fig. 2), which are characterized as *T. cruzi* ligands because their sugar residues readily bind with mammalian host cells (Villalta and Kierszenbaum 1984; Sibley and Andrews 2000). Other adhesion molecules include trans-sialidases and glycoproteins (gp82, gp80, gp35/50, and gp85) (Schenkam et al. 1991). Barrias et al. (2013) suggest any class of molecules exposed on the mammalian host cell surface is potentially a *T. cruzi* receptor ligand.

Cell signaling processes drive the internalization of *T. cruzi* through the formation of an endocytic vacuole (Fig. 3), called a parasitophorous vacuole (Barrias et al. 2013). Lysosomes fuse with microtubules and endosomes, which form an acidic parasitophorous vacuole (Fig. 4); if the phagolysosome fusion does not include endosomes, the parasitophorous vacuole does not mature and does not acidify, thus allowing the parasite to escape (Barrias et al. 2013).

Of the different intracellular entry mechanisms, the most fundamental is phagocytosis, which is an essential mechanism of the innate immune response stimulated by the presence of *T. cruzi* (Barrias et al. 2013). Phagocytosis is an actin-dependent process that is triggered by the interaction of ligands and receptors, stimulating particle internalization (Haglund and Welch 2011). Pattern recognition receptors, or Fc receptors, line the surface of phagocytic cells such as

macrophages and dendritic cells. Once a macrophage binds to a *T. cruzi* trypomastigote, the macrophage phagocytizes the trypomastigote by pseudopodal extensions (Barbosa and Meirelles 1995). The engulfed trypomastigote forms a parasitophorous vacuole with CR3 and Fc receptors, β1 integrin, and lysosomal membrane glycoproteins (Hall et al. 1991). Phagocytosis may occur through the recognition of toll-like receptors 2, although this process also initiates an inflammatory pathway (Manganto-Garcia et al. 2008).

During receptor-mediated endocytosis, clathrin-coated vesicles are formed (Doherty and McMahon 2009). The clathrin coat is constructed of triskelion-shaped molecules which contain three clathrin heavy chains and associated clathrin light chain subunits (Mooren et al. 2012). Clathrin-mediated endocytosis is a standard packaging tool used by the low-density lipoprotein receptor (LDLr) and is required for the internalization of large structures, such as viruses (Mercer et al. 2009; Andersson 2012). The LDLr is involved in the fusion of the parasitophorous vacuole, which promotes the participation of clathrin-coated pits in the entry of *T. cruzi* due to the concentrated LDL receptors in the vesicle (Barrias et al. 2013).

Kleshchenko et al. (2004) describe *T. cruzi* attachment to human galectin-3, which is a member of the β-galactosidase-binding animal lectin family (Souza et al. 2010). Human galectin-3 is a regulator of inflammation, tumor progression, cell growth, and metastasis (Rabinovich et al. 2002; Ochieng et al. 2004), and is expressed on the cell surface in a multitude of tissues and cell types (Gritzmacher et al. 1988). The protein that binds *T. cruzi* to galectin-3 is not yet identified. Trypomastigotes can invade nearly every nucleated cell, and this process is mediated by calcium ions and two proteins found on *T. cruzi*'s membrane including a neuraminidase/trans-sialidase, which binds to sialic acid. The other protein is penetrin, which binds to heparin sulfate (Ortega-Barria and Pereira 1992; Herrera et al. 1994). Additionally, gp82 is required for *T. cruzi*

to penetrate the gastric mucosal epithelium if ingested, as gp82 can bind to gastric mucin and establish an infection (Neira et al. 2003). The parasitophorous vacuole forms from the host cell plasma membrane and this process is facilitated by the depolymerization of the host cell actin microfilaments (Woolsey et al. 2003). Additionally, formation of a phagolysosome, a cytoplasmic body formed by the combination of a phagosome and a lysosome, is required for *T. cruzi* development and replication (Andrade and Andrews 2004). Dynamin is essential to the parasitophorous vacuole formation and entry through endocytosis (Barrias et al. 2010).

After invading the host cell and developing a parasitophorous vacuole, *T. cruzi* employs different escape mechanisms to survive intracellularly. For example, *T. cruzi* neutralizes the pH of the parasitophorous vacuole (pH of 4.5-5), which evades the destruction caused by lysosomal enzymes (Herrera et al. 1994). Additionally, *T. cruzi* produces Chagasin, a cysteine protease inhibitor which aids in avoiding lysosomal derived cysteine protease activity (Santos et al. 2005). *T. cruzi* secretes cruzipain, which induces the upregulation of host-derived arginase-2, and subsequently inhibits apoptosis and extends the life of the infected cell (Aoki and Guinazu 2003). Trypomastigotes secrete trans-sialidase/neuraminidase which removes sialic acid residues from the parasitophorous vacuole (Souza et al. 2010). This makes the parasitophorous vacuole membrane sensitive to the Tc-Tox peptide, which degrades the parasitophorous vacuole membrane and allows trypomastigotes to enter the cytosol, where they transform into amastigotes and divide (Andrews and Whitlow 1989; Andrews et al. 1990; Ley et al. 1990; Aoki and Guinazu 2003). After multiple divisions, some amastigotes transform into trypomastigotes and lyse the host cell, which releases the parasites into the surrounding tissues and bloodstream to infect more cells, notably cells of the central nervous system, cardiac musculature, myenteric plexus, urogenital tract, and the reticuloendothelial system (Rassi et al. 2010).

Triatomines acquire *T. cruzi* when they take a blood meal and ingest circulating trypomastigotes (Rassi et al. 2010). Ingested trypomastigotes travel to the midgut and transform into epimastigotes and divide, thus completing the life cycle.

Acute Chagas Disease Cellular Pathogenesis and Immunity

Once inside the mammalian host, metacyclic trypomastigotes differentiate into trypomastigotes, which initiates the acute mammalian infection (Rassi et al. 2010; Bern et al. 2011). Seventy percent of acute infections are asymptomatic, however some newly infected individuals display characteristic periorbital edema, known as Romana's sign, for up to a week after infection (Bern et al. 2007). Other symptoms may include a chagoma, intermittent fever, hepatosplenomegaly, and inflammatory reactions (Rassi et al. 2010; Barrias et al. 2013). The incubation period following vectorial transmission is up to two weeks, and acute infections often last up to 12 weeks (Bern et al. 2011). Generally, infected individuals survive acute infections and become asymptomatic, however severe hyper-allergic reactions in children living in endemic areas are reported and cases where infection was obtained orally represent severe morbidity and quick death (Valente et al. 2009; Bern et al. 2011; Xavier et al. 2014).

During an acute infection, a host immune response is mounted against the parasite, which reduces parasitemia to a subpatent concentration; however, without antitrypanosomal drugs such as nifurtimox and benznidazole, *T. cruzi* is not eradicated from the host and often persists in myocytes and enteric ganglia indefinitely, signaling the transition to a chronic infection (Rassi et al. 2010). To avoid immune detection, trypomastigotes can coat their membrane with surface proteins from lysed cells (Rassi et al. 2010). Diagnosis and treatment are most effective during an acute infection.

Intracellular amastigotes replicate for 4-5 days before reverting to trypomastigotes which lyse the cell, infect adjacent cells, and enter the bloodstream to infect new areas and cause systemic infection (Rassi et al. 2000, 2010). Circulating trypomastigotes disseminate by penetrating cardiac smooth, and skeletal myocytes; neurons; lymph nodes; hepatocytes; adipocytes; bone marrow; adrenals; and the spleen (Shoemaker et al. 1970; Rassi et al. 2000).

T. cruzi infections cause partial immunosuppression that favors the survival of the parasite within the vertebrate host (Sher and Snary 1985). This was observed *in vitro* through human dendritic cells that were experimentally infected with *T. cruzi*, which down-regulated the synthesis of interleukin 6 and 12, tumor necrosis factor α, HLA-DR, CD-40. This process halted the maturation of dendritic cells into antigen recognition cells (Overtvelt et al. 1999). *T. cruzi* amastigotes utilize calreticulin, which aids in calcium trafficking and storage (Ferreira et al. 2004). During an acute infection, *T. cruzi* trypomastigotes and the host immunoinflammatory response typically causes minor tissue damage (Andrade 1999).

While circulating in the bloodstream, trypomastigotes shield themselves from host defenses by coating themselves in a complement regulatory protein that binds the C3b and C4b components, thus stopping the alternate pathway (Beucher et al. 2003). Vertebrate host immunity can develop and serum antibodies are ultimately produced against circulating trypomastigotes. Host immunity is contingent on CD1d antigen presentation and upregulation of interleukin 12 to produce natural killer cells, which are effective against encysted amastigotes (Duthie et al. 2005). Amastigotes are killed by the production and influx of nitric oxide and nitric oxide synthase, which are potent trypanocides (Vespa et al. 1994). Although *T. cruzi* is not eliminated unless treated, CD8+ T cells recognize the parasite antigens and minimize parasitemia (Martin and Tarleton 2005).

Chronic Chagas Disease Cellular Pathogenesis and Immunity

As previously mentioned, *T. cruzi* invades multiple tissues and organs, and untreated individuals remain infected indefinitely. Amastigotes invade the myenteric plexus and cause space occupying cysts that disrupt nerve impulses, reduce muscle tone, and enlarge areas of the digestive tract (Bern 2011). This enlargement is called megacolon and megaesophagus and is characteristic of late sequelae and chronic infection. Destruction of intramural autonomic ganglia, which affects the esophagus and colon, leads to chronic Chagas gastrointestinal disease (Koberle 1968). Esophageal manifestations vary from asymptomatic motility disorders to achalasia, to megaesophagus (Oliveira et al. 1998). Symptomatology may include dysphagia, odynophagia, esophageal reflux, weight loss and malnutrition, aspiration, ptyalism, cough, and regurgitation (Rassi et al. 2010; Bern et al. 2011). Individuals with achalasia are more likely to develop esophageal carcinomas (Brucher et al. 2001). Megacolon is characterized by constipation and may cause fecaloma, volvulus, abdominal distension, and bowel ischemia (Rassi et al. 2010; Bern et al. 2011).

Marked cardiomyopathy is synonymous with chronic *T. cruzi* infections. In the 40% of individuals that develop clinical Chagas disease, heart tissue is eroded and leads to aneurysms and heart failure (Rassi et al. 2010). The causes of Chagas cardiomyopathy are similar to myenteric plexus disease, but the presence of *T. cruzi* in the heart also causes fibrosis which is a dominant factor of heart damage (Machado et al. 2012). In chronic infections, disease morbidity is balanced by immune-mediated parasite containment and damaging inflammatory responses (Rassi et al. 2010). It is unknown whether myocardial tissue damage is caused directly by parasite factors, or indirectly through parasite-driven immunopathology. However, a persistent *T. cruzi* infection is required for most cardiac manifestations (Rassi et al. 2010).

In chronic Chagas cardiomyopathy, low intensity and slowly progressive tissue invasion impairs contractile function and results in general dilation of all four chambers (Rassi et al. 2010). A marked left ventricular apical aneurysm and wall dysfunctions are typical and manifest in early infections (Acquatella 2007). Histologically, myocardial cell destruction, diffuse fibrosis, edema, myocardium infiltration by mononuclear cells, and fibrotic tissues scars are observed (Andrade 1983). Atrioventricular blockage, intraventricular blockage, and sinus node dysfunction result from myocarditis. Continual cardiac fiber destruction and fibrosis cause heart failure and ventricular arrhythmias (Rassi et al. 2000). Further myocardial damage stems from abnormalities in the coronary microvasculature and focal hypoperfusion (Rossi 1990). Systemic and pulmonary embolisms occur from mural thrombi in the cardiac chambers (Oliveira et al. 1983). Embolisms are found in the lungs, kidneys, spleen, and brain (Rassi et al. 2010). Late manifestations of Chagas cardiomyopathy include ventricular extrasystoles, ventricular tachycardia, bradycardia, thromboembolic occurrences, and congestive heart failure (Rassi et al. 2000). This causes palpitations, presyncope, syncope, and a high risk of sudden death (Rassi et al. 2007).

Chagas Disease Treatment

Like other neglected tropical diseases, there is no vaccine available to prevent an infection with *T. cruzi* and treatment options are limited to two drugs discovered over 30 years ago (Boiani et al. 2010). Treatment options include two antitrypanosomal drugs, nifurtimox and benznidazole. The United States FDA only approves benznidazole, and both are limited in the United States (Bern et al. 2007). Both drugs are nitroheterocycle compounds. A heterocycle is a cyclic compound that contains two different elements within its rings, and the compound has some derivative of Nitrogen, such as nitrofurane or nitroimidazole (Boiani et al. 2010). Like

other nitrocompounds, it is believed that benznidazole and nifurtimox apply their biological activity through the bioreduction of their nitro group (Maya et al. 2007). This process starts by reducing the nitro group to a nitro anion radical through a reaction catalyzed by a putative NADPH/NADH nitroreductase (Maya et al. 2007). For Nifurtimox, the nitro anion radical undergoes redox-cycling with molecular oxygen, thus yielding superoxide anion (O_2^-) and subsequently hydrogen peroxide (H_20_2) through a superoxide dismutase catalyzed reaction (Boiani et al. 2010). In the presence of iron, these species form a strong oxidant hydroxyl radical by a Haber-Weiss reaction. The increased production of a reactive oxidant species causes oxidative stress in the parasite that weakens endogenous anti-oxidant enzymes (Boiani et al. 2010). Benznidazole treatment lasts for 60 days and is preferred due to the better tolerance of side effects and higher efficacy against trypomastigotes and amastigotes. Both drugs are mutagenic and have increased lymphomas in animal testing (Bern et al. 2007). Benznidazole disrupts protein, RNA, and DNA synthesis; damages nuclear and kinetoplast DNA, and causes macromolecule degradation (Polak and Richle 1978; Goijman and Stoppani 1985; Goijman et al. 1985; Rajao et al. 2013). 8-oxoguanine and oxidized deoxyguanosine triphosphate (8-oxodGTP) cause double-strand DNA breaks in *T. cruzi* (Rajao et al. 2013). Drugs have limited efficacy in chronic cases due to inadequate pharmacokinetics (Urbina 2003).

Genetic Lineages of Trypanosoma cruzi

Memorias do Instituto Oswaldo Cruz (1999) details the two main lineages of *T. cruzi*: *T. cruzi* I (TcI) predominates in sylvatic transmission cycles, is less resistant to trypanocides, and is associated with human disease in all endemic countries found north of the Amazon basin. Evidence suggests this lineage coevolved with arboreal opossums and vectors within the tribe Rhodniini (Gaunt and Miles 2000). TcI is currently the only reported lineage found in humans in

North America (Roellig et al. 2008). Based on multilocus microsatellites, single nucleotide polymorphisms, and four recognized haplotypes, sublineages TcIa to TcId were proposed. Haplotypes TcIa and TcIc were associated with humans and domiciliated vectors, whereas haplotypes TcIb and TcId were exclusive to sylvatic transmission cycles (Herrera et al. 2007). Although these differences were recognized, the TcI lineage is not subdivided into sublineages (Bern et al. 2011).

The second lineage, *T. cruzi* II (TcII) predominates domestic cycles through South America, has elevated resistance to trypanocides, and is related to tissue damage caused by Chagas disease (Di Noia et al. 2002; Freitas et al. 2005). The TcII lineage originally was subdivided into five discrete typing units: IIa, IIb, IIc, IId, and IIe (Brisse et al. 2000). For simplicity, parasite strains were later renamed TcI, TcII, TcIII, TcIV, TcV, and TcVI and are called discrete typing units (DTUs; Zingales et al. 2009; Pinto 2015). Strains TcI and TcII are ancestral strains, and TcV and TcVI arose from hybridization events (Zingales et al. 2009). The origins of strains TcIII and TcIV are still undetermined. TcI and TcII diverged somewhere between 88 and 37 million years ago (Machado and Ayala 2001). Size polymorphism was used to classify genotypes and sequence analyses of multiple gene loci, including the miniexon gene, intergenic region of the miniexon gene, the 18S rRNA gene, the 24Sα rRNA gene, internal transcribed spacer regions, and different housekeeping genes (Westenberger et al. 2006).

Lineage TcIII coevolved between burrowing xenarthrans, such as armadillos, and triatomines in the Triatomini tribe (Yeo et al. 2005). About 65 million years ago, xenarthrans and marsupials were among the first mammalian inhabitants of South America, predating primates, rodents, and bats by 40 million years. (Gaunt and Miles 2000). TcIII is distributed throughout

South America and is predominately sylvatic. Shared hosts are armadillos, terrestrial marsupials, rodents, and skunks (Yeo et al. 2005).

Lineages TcII, TcV, and TcVI frequently are reported in South American human Chagas disease (Miles et al. 2009). These lineages are closely tied to the domestic transmission cycle and the domestic vector *Triatoma infestans*. TcV and TcVI are common among cardiomyopathy and intestinal megasyndromes along the southern cone (Miles et al. 2009). TcV is most commonly associated with congenital infections (Miles et al. 1981). Additionally, Freitas et al. (2006) discovered a *T. cruzi* III lineage (TcIII).

TcI and TcIV are the only two lineages reported from mammals and vectors in the United States (Roellig et al. 2008; Curtis-Robles et al. 2016). In raccoons, TcIV predominates, however, there is little cardiac pathology within wild carnivores (Bern et al. 2011; Curtis-Robles et al. 2016). TcI and TcIV are reported from nine-banded armadillos, domestic dogs, and macaques (Roellig et al. 2008). Interestingly, all reported autochthonous infections in the United States are TcI lineage (Roellig et al. 2008).

Trypanosoma cruzi in the United States

The first detection of *T. cruzi* in the United States was in a triatomine collected from San Diego, California in 1916 (Kofoid and McCulloch 1916; Bice 1965). *T. cruzi* is reported across the central and southern U.S., from Florida to California and northward to Maryland along the east coast, with several established enzootic cycles where sylvatic transmission predominates (Hernandez et al. 2016; CDC 2017 https://www.cdc.gov/parasites/chagas/gen_info/detailed.html). These cycles involve multiple triatomine species and 150 mammalian reservoir hosts, such as domestic and wild dogs, raccoons, opossums, armadillos, woodrats, livestock, and bats (Hoare 1972; Anonyme 1991;

Anez et al. 2009; Bern et al. 2011; Bezerra et al. 2014; Hodo et al. 2016; Nichols et al. 2018). Historically, endemic *T. cruzi* transmission was not reported in the United States; however, increasing reports of autochthonous vectorial transmission suggest both endemic and enzootic transmission cycles occur in the United States (Dorn et al. 2007; Curtis-Robles et al. 2015, 2016; Hernandez et al. 2016; Gunter et al. 2017).

Although *T. cruzi* endemicity in the United States is still being evaluated, living conditions in the United States vary from South American countries endemic with *T. cruzi*. Living conditions such as thatched roofs and cracked walls in adobe houses are ideal triatomine habitats and facilitate peridomestic colonization (Lent and Wygodzinsky 1979; Bern et al. 2011). These factors coupled with the presence of infected domestic animals significantly increase transmission risk to humans in endemic areas (Bezerra et al. 2014). In the United States, several factors hinder endemic *T. cruzi* cycles, such as improved housing conditions, the predominant sylvatic transmission cycle, which is often restricted to enzootic transmission and reduces human exposure, and delayed triatomine defecation times post-feeding (Grundemann 1947; Maurer 2013 https://animaldiversity.org/accounts/Triatoma_sanguisuga/#372FAABF-7551-431A-BF73-21A6BCA3AEA6; Hernandez et al. 2016). Other factors that play a role in the rarity of Chagas disease in humans in the United States are scarcer domestic dwellings for triatomines, triatomine zoophilicity, low virulence of indigenous strains, and possible misdiagnosis (Kagan et al. 1966; Zeledon 1974; Milei et al. 1992; Pung et al. 1995).

Trypanosoma cruzi in Oklahoma, USA

The first incidence of *T. cruzi* in Oklahoma was reported from wild raccoons in Tulsa (John and Hoppe 1986). The second and third reports were from domestic and wild dogs in Bartlesville, Nowata County, LeFlore County, and Pittsburg County (Fox et al. 1986; Bradley et

al. 2000). The fourth incidence of *T. cruzi* in Oklahoma was reported in a juvenile Mexican free-tailed bat (*Tadarida brasiliensis*) at a maternity roost in Woodward County (Nichols et al. 2018). The first three enzootic reports are likely autochthonous because *Triatoma sanguisuga* and *T. lecticularia* are found statewide, although no triatomines were sampled in these reports. The parasite's genetic sequences from the fourth report closely matched numerous reports from south Texas, which suggest the mother of the infected Mexican free-tailed bat likely obtained the infection while migrating through Texas.

Triatomine Vector Biology and Ecology

Triatomines belong to the subfamily Triatominae (Hemiptera: Reduviidae: Triatominae) and are commonly called kissing bugs. There are more than 130 triatomine species distributed across the Americas, most of which can be infected by and transmit *T. cruzi* (Lent and Wygodzinsky 1979). Triatomines are nocturnal and hematophagous, requiring a blood meal to develop through each of the five nymphal stages. This behavior leads to infected nymphs and adults. Both males and females are hematophagous, and females require a blood meal to lay eggs. Most triatomine species acquire a blood meal without waking the host through the aestheticizing action of the bug's saliva (Lent and Wygodzinsky 1979). A blood feeding can last up to 30 minutes, and triatomines often bite around mucosal areas, such as the lips or eyes (Lent and Wygodzinsky 1979). Triatomines are specialized for a hematophagous lifestyle by utilizing a characteristically robust proboscis. During a blood meal, triatomines generally defecate onto the host immediately, or within minutes of feeding (Lent and Wygodzinsky 1979). Triatomines can survive several months without a blood meal and survive winters by reducing activities (Lent and Wygodzinsky 1979).

Two transmission cycles are observed, sylvatic and domestic. Sylvatic triatomines colonize the nests of small mammals or marsupial hosts (Lent and Wygodzinsky 1979). There are reports of sylvatic triatomine adults that have flown into human dwellings and caused sporadic human infections (Coura et al. 2002). Nest colonization is achieved through mammalian heat signatures, mammalian odors, water vapor, and pheromones released in triatomine feces, which attract other triatomines (Lorenzo and Lazzari 1996; Claudio et al. 2013). Inside a nesting area, triatomines exhibit negative phototaxis, although they can be photophilic at night (Claudio et al. 2013). Endogenous and exogenous mechanisms maintain akinesia during daytime hours because circadian clocks reduce the overall activity of the triatomines (Lazzari 1992). Thigmotaxis is a mechanosensory stimulation by physical contact with congeners and the substrate to which the triatomines are contactual (Wigglesworth and Gillet 1934). Heat is one of the most potent physical cues for triatomines. Triatomines have evolved a heat gradient detection ability and use this to locate and feed on blood vessels (Ferreira et al. 2007). Lorenzo et al. (1999) demonstrated that *T. infestans* could detect the heat emitted by a human face from two meters away and the heat from a dog from several meters away. Triatomines can maintain high *Trypanosoma* spp diversity within their ecological niches, especially when they routinely feed on bats (Dario et al. 2017).

Domestic transmission is clinically significant and is characterized by triatomine vectors that are adapted to living in human houses and animal enclosures, and infecting domesticated species such as dogs, cats, guinea pigs, and chickens (Herrer 1964). Domestic habitation ensures abundant blood meal sources, and in areas with poor quality housing, triatomines readily colonize cracks and crevices of walls and thatched roofing (Levy et al. 2006). In endemic countries, 25% to 100% of homes may be infested, and the surrounding areas house large

colonies of juvenile and adult insects (Levy et al. 2006). Some peridomestic triatomines aggressively pursue their potential hosts with an extended proboscis (Gurtler et al. 2009; Bezerra et al. 2014).

Miles et al. (2003) reported that some triatomine vectors have adapted to invade both domestic and sylvatic locations, and aid in connecting the domestic and sylvatic cycles. Two species of triatomines in Oklahoma are reported, *Triatoma lecticularia* and *T. sanguisuga* (Usinger 1944; Drew and Schaefer 1962). These two species currently are observed in sylvatic cycles. Triatomine species that colonize domestic and peridomestic environments increase the epidemiologic risk to humans and livestock. The epidemiologic risk is related to the coevolved parasite/vector biology, ecological variables of triatomine species, and vector competence. Some of the important species in Latin America are *T. infestans, T. dimidiate, T. brasiliensis, Rhodnius prolixus,* and *Panstrongylus megistus* (Pan American Health Organization 2014 https://www.paho.org/hq/index.php?option=com_topics&view=article&id=10&Itemid=40743&lang=en). The important species in the United States are *T. sanguisuga, T. lecticularia, T. gerstaeckeri, T. indictivia,* and *T. protracta* (Bern et al. 2011). Early examination of *T. sanguisuga* and *T. protracta* infection rates varied from 6% to 20% (Usinger 1944; Kagan et al. 1966). Recent infection reports are considerably higher, with 51% and 63% in Texas triatomines (Kjos et al. 2009; Curtis-Robles et al. 2016). *Triatoma sanguisuga* is distributed countrywide and may be the most important vector in the United States (Lent and Wygodzinsky 1979; CDC 2017 https://www.cdc.gov/parasites/chagas/gen_info/detailed.html).

Triatoma sanguisuga completes eight nymphal instars stages before maturing into an adult (Grundemann 1947). This species can live up to three years, hibernate during the winter, and survive 100 days on only three blood meals (Grundemann 1947). Adults are phototactic,

whereas nymphs are photophobic (Grundemann 1947). *T. sanguisuga* are gregarious and are active from the spring months to late fall. They preferentially feed nocturnally and contain an anesthetic in their saliva, although some allergic reactions and acute pruritis occur, possibly increasing the risk of host self-inoculation with *T. cruzi* laden feces (Griffith 1947; Nichols and Green 1963; Klotz et al. 2010). *T. sanguisuga* typically feed unnoticed between three to eight minutes. While engorged, *T. sanguisuga* empties its fecal pouch and excretes a liquid material that contains water and Malpighian tubule residue. Later excretions consist of dried darker materials with considerable quantities of hemoglobin (Grundemann 1947). During the excretions, infected triatomines unintentionally release small amounts of metacyclic trypomastigotes. Molting hormones are released post feeding and initiate ecdysis. While digesting a blood meal, females deposit eggs in their nests, which are commonly found within woodrat nests (Grundemann 1947). *T. sanguisuga* also is known to transmit equine encephalomyelitis virus (Kansas State College of Agriculture and Applied Science 1940). Interestingly, Charles Darwin may have been infected from triatomines and developed Chagas disease, as documented in his *Voyage of the Beagle* journal (Campbell and Matthew 2005).

Triatomine species vary in host preference and some habitually feed on reptiles and birds, which are reported to harbor *Trypanosoma* spp such as *T. cascavelli*, *T. serpentis*, *T. avim*, and *T. culicavium*, although reports suggest the ingestion of infected triatomines is a common infection route (Viola et al. 2009; Votypka et al. 2012; Curtis-Robles et al. 2016). This behavior can potentially influence the future diversity of trypanosome species. Triatomines are the classical vectors of *T. cruzi*, however, other arthropods may potentially transmit *T. cruzi*. A novel avian trypanosome, *T. culicavium* was isolated from *Culex* mosquitoes (Votypka et al. 2012). The authors noted the transmission route of *T. culicavium* was achieved exclusively by ingestion of

infected *Culex* mosquitoes and the mosquitoes were unable to transmit the parasite while obtaining a blood meal. Bed bugs (*Cimex lectularius*) experimentally harbored and transmitted *T. cruzi* to laboratory mice (Salazar et al. 2015). Sandflies (*Phlebotomus* spp) can transmit reptilian trypanosomes to an uninfected host via ingestion (Anderson and Ayala 1968). There are currently no published records of the potential phoresy of a triatomine by a mammal, although this potential should be investigated.

Bats as Natural Reservoirs of Zoonoses

Chiropterans, commonly called bats, are the only mammals capable of powered flight. This unique ability enables bats to inhabit a multitude of ecological regions and traverse between different ecosystems. Chiropterans are the most diverse and geographically distributed mammalian taxon, found on all continents except Antarctica (Schipper et al. 2008). The 1,100 species of bats constitute roughly 20% of current known mammalian species and are only outnumbered by members of the order Rodentia (Schipper et al. 2008). Bats are a keystone species of the global ecosystem and humans benefit from their presence in multiple ways (Allocat et al. 2016). The roles of bats include seed dispersal, pollination activities, predation of nocturnal insects such as crop and human pests, and the production of nitrogen rich guano which makes an excellent biological fertilizer (Allocat et al. 2016).

Despite the beneficial behavior of bats, there exists an involuntary danger to humans. Bats are natural reservoir hosts and sentinels of infection for numerous pathogenic microorganisms, many of which cause severe human diseases, are continually linked to zoonotic virus outbreaks, and host a significantly higher proportion of zoonoses than any other mammalian order (Calisher et al. 2006; Brook and Dobson 2015; Olival et al. 2017). Human-bat interactions occur because peridomestic bats are dispersed in urban regions. As a result, they can

enter areas where domestic animals and humans inhabit and contaminate houses with guano and urine (Allocat et al. 2016). However, it is not the expansion of bat populations, but instead the encroachment of humans into bat habitats through deforestation and habitat fragmentation which can put humans at risk for zoonoses (Hayman et al. 2013).

Aggregation in large roosts promotes microbial transmission within bat colonies (Allocat et al. 2016). Bats can then transmit infectious pathogens to humans through intermediate hosts or direct contact. Intermediate hosts may be infected through different routes, notably through ingesting partially digested food passed from bats, and subsequently come into direct contact with humans (Allocat et al. 2016). Frugivorous bats have a select diet that favors the aerodynamics of flight, therefore they obtain nutrients by chewing fruits and then spitting the residues (Dobson 2005). Potentially infectious residue can be dropped onto the ground and subsequently ingested by other animals. This method is also described for viral transmission from insectivorous bats (Dobson 2005). Direct contact between humans and bats can promote infection in regions where humans cook and eat bats, deforestation increases human-bat encounters, or through the bite of rabid bats (Han et al. 2015).

Other characteristics such as hibernation, longevity, migration, and flight promote infectious pathogen persistence. Hibernation may influence pathogen maintenance during cold weather, such as *Pseudogymnoascus destructans*, the etiological agent of White Nose Syndrome (Allocat et al. 2016). The longevity of many bats can reach 30 years, which is much longer than many rodent species, and enables bats to maintain infections in their environment for decades. Migration enables some bat species to traverse thousands of kilometers annually and spread pathogens across far distances as well as acquire new pathogens (Krauel and McCracken 2013; McGuire et al. 2013). Interestingly, many pathogens are not pathogenic against bats and persist

for extended periods without killing the host (Allocat et al. 2016). Over 200 viruses are associated with bats, and despite being infected with more different zoonotic viruses per any other host, except rabies and other *Lyssaviruses*, the viruses are not pathogenic for the infected bat (Allocat et al. 2016). This suggests bats may control viral replication more efficiently than other mammalian hosts, which may be due to mechanisms associated with flight. During flight, bats increase their metabolic rate and body temperature comparable to that of a fever response. This renders the replication of infectious pathogens, which are temperature sensitive, less favorable (O'Shea et al. 2014).

Bats as the Primary Reservoir of American Trypanosoma Species

Phylogenetic analyses of 18S rRNA sequences suggests that salivarian trypanosomes (the African *T. brucei* clade which are transmitted by bites) diverged from the stercorarian trypanosomes (the American *T. cruzi* clade which are transmitted by contaminated feces) approximately 100 million years ago (Stevens et al. 1999). During this time, South America, Antarctica, and Australia separated from Africa and *Trypanosoma* species moved to new regions (Hamilton et al. 2012a). Molecular evidence suggests *T. cruzi* evolved from an ancestral bat trypanosome, which supports the bat seeding hypothesis (Hamilton et al. 2012a, 2012b). This hypothesis suggests that trypanosome infected bats colonize regions of South America, share the same ecotopes with terrestrial mammals and triatomines, and through their great dispersal patterns and migratory pathways, bats are a platform for *T. cruzi* to emerge in novel foci. The closest genetically characterized relative of *T. cruzi*, *T. marinkellei*, is found readily in South American bats (Stevens et al. 1999; Hamilton et al. 2004, 2007; Cavazzana et al. 2010; Dario et al. 2017). Both trypanosomes diverged from an ancestral species between 6.5-8.5 million years ago (Machado and Ayala 2001; Lewis et al. 2011), although *T. marinkellei* may be a subspecies

of *T. cruzi* (Baker et al. 1978). The recently described *T. erneyi* and *T. livingstonei* found in bats from Mozambique and *T. dionisii* from Old and New World bats are also close relatives of *T. cruzi* (Stevens et al. 1999; Hamilton et al. 2007, 2012b; Cavazzana et al. 2010; Lima et al. 2012; Lima et al. 2013). Additionally, *T. cruzi* has been detected in South American bats with one specific DTU, TcBat, currently found only has only in bats (Lisboa et al. 2008; Marcili et al. 2009; Cavazzana et al. 2010; Ramirez et al. 2014). TcBat is most closely related to *T. cruzi* TcI, which in South America is associated with opossums and kissing bugs within the genus *Rhodnius* in arboreal environments (Hamilton et al. 2012a). These supporting claims suggest the common ancestor of the *T. cruzi* clade was a bat trypanosome.

Trypanosome infected bats colonized South America between 7-10 million years ago (Stadelmann et al. 2007). Subsequently, different independent bat trypanosome lineages switched from bats into terrestrial mammals through triatomine vectors feeding on both bats and terrestrial mammals within the same arboreal ecotope (Stevens et al. 1999). One report suggests *T. cruzi* rose in the Pliocene by repeatedly passing between different mammalian hosts (Flores-Lopez and Machado 2011). This report also suggests *T. cruzi* diversified into the current DTU lineages, TcI-TcVI and TcBat about 1-3 million years ago (Flores-Lopez and Machado 2011).

Recent data confirms bats are the primary reservoir hosts of *T. c. cruzi* in Brazil and supports bats as suitable reservoir hosts for several *T. cruzi* discrete typing units, as well as many different *Trypanosoma* species, which can occur in single or mixed infections (Dario et al. 2017). Bat trypanosomes in the *T. cruzi* clade are morphologically indistinguishable and may represent an undiscovered world of trypanosomatids (Hoare 1972).

Tadarida brasiliensis

Tadarida brasiliensis, Mexican free-tailed bats (MFT), is a migratory species within the Order Chiroptera and Family Molossidae. MFT migrate annually from as far south as Argentina to as far north as Ohio, giving this species one of the largest mammalian geographic ranges in the western hemisphere (Fig. 5; Wilkins 1989). The northern distribution extends along Oregon, Nevada, Ohio, Arkansas, Alabama, Mississippi, Georgia, and North Carolina (Lee and Marsh 1978; Wilkins 1989). Due to seasonal differences, MFT populations east of Texas (*T. b. cynocephala*) and populations of *T. b. mexicana* between Oregon and California do not migrate but instead show seasonal movements (Cockrum 1969; Lee and Marsh 1978). Populations of *T. b. mexicana* in the Central United States summer in the Great Plains and migrate southward into southern Texas and Mexico (Glass 1982).

During the spring and summer, Oklahoma is home to three million MFT (Oklahoma Department of Wildlife Conservation 2013). Mexican free-tails are so prevalent in Oklahoma that they are the state flying mammal (Oklahoma Department of Wildlife Conservation 2013). MFT are called the jets of the bat world, due to their ability to fly quickly and for long distances. The primary flight muscles (pectoralis, subscapularis, and serratus ventralis) of the MFT are composed of fast oxidative fibers, which indicate their design is for high speed flight over extended periods of time (Foehring and Hermanson 1984). A combination of fast oxidative and slow oxidative fibers constitute the accessory flight muscles (triceps brachii and biceps brachii). Deep, slow oxidative fibers likely stabilize the wings, whereas fast oxidative fibers flex and extend the humerus and aid humeral adduction during the downstroke (Foehring and Hermanson 1984). The primary form of lactate dehydrogenase in the pectoralis muscle is B_4 and is commonly found in species capable of sustained flight (Gutierrez et al. 1974; Wilkins 1989).

MFT aggregate in North American maternity roosts in the spring consisting of caves, abandoned buildings, or underneath bridges, and synchronically emerge at dusk to feed (Wilkins 1989). Initially, maternity roosts are exclusive to pregnant females (Cockrum 1969). Typically, several males do not migrate into North America during the spring and remain in bachelor colonies year-round (Glass 1982). Many males within a bachelor colony are offspring from that birthing season (Glass 1982). Some males migrate into North America and share bachelor colonies, but those number only a few hundred males (Cockrum 1969; Hermanson and Wilkins 1986). Both males and females use transient roosts during migration (Cockrum 1969).

While roosting, MFT employ physiological mechanisms to counter high atmospheric concentrations of ammonia found in their roosts. MFT are known for their large guano deposits, up to 99 metric tons of guano per cave in Texas (Osborne 1939). MFT can filter 97.1% of the ammonia in an inhaled mixture of 1,130 ppm, 73.4% at 4,420 ppm, 72.6% at 5,040 ppm, and 77.5% at 72,00 ppm (Studier 1969). Interestingly, ammonia filtration is passive, as indicated by decreasing metabolic rates with increasing ammonia concentrations (Studier et al. 1967). MFT exhibit a swarming behavior inside roosts and this is hypothesized as a method of wafting ammonia laden air out of a roost (Henshaw 1960). There are three reported MFT maternity caves in Oklahoma that house an average of 205,198 bats annually (Ganow et al. 2013). Mexican free-tails inhabit these roosts from early spring through the fall before migrating south along the Sierra Madre Oriental into south-central Mexico for the winter (Glass 1982; Ganow et al. 2013).

MFT are highly gregarious and insectivorous, with a colony eating up to 18,000 metric tons of insects annually (Allen et al. 2008). The diet of the MFT is 90% moths, primarily Gelechiid moths (Ross 1961). Insect predation is enabled by echolocation, and this ability is vital for flight and may allow for a mother to identify her pup when returning from a nightly meal

(Wilkins 1989; Allen et al. 2008). MFT often fly 50 km or more to reach foraging areas. Flight to the foraging area is rapid, direct, and usually involves gliding (Davis et al. 1962). Many MFT fly over 40 km/h and as high as 3,000 meters (Williams et al. 1973). The majority of foraging is at heights between six and 15 meters (Caire et al. 1984).

MFT harbor numerous pathogens, such as the rabies virus and *Histoplasma capsulatum*, and various helminths, protozoa, and ectoparasites, and act as a natural reservoir for trypanosomes (Bryles et al. 1969; Ubelaker 1970; Pinto et al. 2011; Hamilton et al. 2012a; Hodo et al. 2016). I suspect MFT contributes to the enzootic emergence of *T. cruzi* in Oklahoma by their annual migration from endemic Central and South America to North American maternity roosts. Previous studies sampled various chiropterans for *T. cruzi* across the Americas (Ubelaker 1970; Anez et al. 2009; Hodo et al. 2016; Dario et al. 2017). The first confirmed report of *T. cruzi* in a bat within the United States was found in one peridomestic *Nycticeius humeralis*, in Texas (Hodo et al. 2016). The DTU of the infected *Nycticeius humeralis* was TcI (Hodo et al. 2016). The authors also found *T. dionisii* in peridomestic MFT.

Study Area

Gypsum caves are abundant in Northwest Oklahoma and are a preferred habitat for Oklahoma MFT (Fig. 6; Ganow et al. 2013). Underneath the gypsum hills lie extensive underground tunnels and multiple caves providing ideal roosting sites for the MFT. Three major maternity roosts are found within Woodward, Woods, and Major Counties, OK. The combined population of the three roosts is estimated to be 205,198 (Ganow et al. 2013).

Field Collection

Sweep netting is a standard collection method for entomological studies; however, it is less frequent for collecting chiropterans. Sweep netting is useful to collect a small number of

emerging chiropterans, as small number can be collected during each sweep. This is beneficial compared to mist netting, in which a significant number of chiropterans would be trapped in the net simultaneously and potentially cause unnecessary stress.

For data records, adult bats can be separated from juveniles by examining the metacarpal-phalangeal joint ossification (Rossinni and Wilkinson 2009). The metacarpal-phalangeal joint of juveniles is less knobby and more evenly tapered than adults. This can be determined by transilluminating the wing using a headlight to visualize the epiphyseal fusion (Rossinni and Wilkinson 2009).

Tissue sampling, such as wing punching, is a safe and universal method for DNA extraction from bats (Weaver et al. 2009; American Museum and Natural History Wing Punch Protocol 2018 http://research.amnh.org/vz/mammalogy/donating-bat-tissue-and-hair-samples-genomic-and-stable-isotope-studies/wing-punch-and-hair-sampling). Wing tissue punches can be obtained from a bat's uropatagium and plagiopatagium without causing permanent damage to the bat and the hole left in the patagium will regrow within 33 days and does not impair flight or metabolism (Weaver et al. 2009). This sampling method is quick and minimizes stress on the bat, as opposed to obtaining blood samples, which are more difficult and hazardous to transport. Vasculature wing tissue punches can test for both amastigotes and trypomastigotes, whereas blood sampling can only test for trypomastigotes and antibodies against trypomastigotes. Additionally, obtaining enough blood for PCR amplification can weaken the bat. Instead of freezing tissues in liquid nitrogen, tissues can be stored in different transport media, such as ATL lysis buffer. ATL is a tissue lysis buffer used in the first steps of the DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland, USA) to break down the sampled tissue. Tissues can be

stored in ATL lysis buffer and proteinase K at room temperature for up to six months without any DNA degradation.

Techniques to Detect Parasitemia

Traditionally, the gold standard for quick and accurate diagnosis is light microscopy of Giemsa stained blood smears; however, this is most effective during acute infection (WHO Expert Committee 2002). Serology tests and polymerase chain reaction (PCR) can detect trace amounts of *T. cruzi* in blood samples and can aid in diagnosing current and historic infections (Bern et al. 2011).

Currently, the options for diagnosing *T. cruzi* infections are serology tests, such as immunofluorescence assays and Enzyme-Linked Immunosorbent Assays (ELISA) that detect IgG antibodies, xenodiagnosis, hemoculture, and PCR (Almeida et al. 1997; Bern et al. 2011). Many serology tests offer high specificity, up to 98%, but often cross-react with other *Trypanosoma* species, such as *T. rangeli* which is a common and nonpathogenic species (Gilber et al. 2013). Also, the CDC recommends using at least two different serology tests for diagnosis, followed by PCR. Xenodiagnosis is uncommon in the United States but can be implemented if serology and PCR methods are unavailable. This method involves raising non-infected triatomines in a lab, then allowing the vectors to feed on a suspect host and analyzing the gut contents for epimastigotes via light microscopy (Pless et al. 1992). This method is not favorable due to the potential risk for a lab-raised triatomine to be accidentally infected if poor lab techniques are practiced (Pless et al. 1992).

PCR is the most reliable tool for diagnosing *T. cruzi* infections (Virreira et al. 2003; Bern et al. 2011). PCR can detect specific *T. cruzi* lineages using primers that amplify a target *Trypanosoma* species and do not amplify other trypanosomatids, such as *Leishmania spp* and the

African trypanosomes (Moser et al. 1989; Virreira et al. 2003; Trejo 2006). Primers, such as TCZ1/TCZ2, are designed to amplify *T. cruzi* nuclear DNA (nDNA) and will not amplify any other *Trypanosoma* species, including nonpathogenic *T. rangeli* DNA (Virreira et al. 2003). PCR can also detect a *T. cruzi* infection even if serology test results are negative (Gilber et al. 2013). PCR is sensitive enough to detect one *T. cruzi* trypomastigote per 20 mL of blood (Sabino et al. 2015); although most studies suggest PCR sensitivity is closer to one parasite per 100 µL of blood (Moser et al. 1989; Virreira et al. 2003; Eloy and Lucheis 2012). One distinct advantage of using PCR for parasitic detection is the ability to analyze both tissues and blood samples, whereas serology tests are designed for blood samples (Virreira et al. 2003; Trejo 2006; Bern et al. 2011; Gilber et al. 2013).

TCZ1/TCZ2 prime a 195 base-pair satellite repeat in the nuclear DNA. This repeat constitutes 9% of the *T. cruzi* genome and is repeated roughly 120,000 times (Gonzalez et al. 1984; Moser et al. 1989). This amplified region is located within the miniexon gene, which presents a chromosomal location that is relatively conserved among species within this genus and shows distinct nucleotide sizes and sequences among closely related species (Eloy and Lucheis 2012). TCZ1/TCZ2 can amplify *T. c. cruzi* and *T. c. marinkellei*, which is a bat associated trypanosome that infects humans (Franzen et al. 2012).

TCZ1/TCZ2 are a better choice than kDNA minicircle (kinetoplast DNA) primers due to the highly conserved and shared 195 base-pair sequence among *T. cruzi* species. Additionally, there are 1.8 times as many copies of the 195 base-pair repeat per organisms as there are of the amplifiable minicircle constant regions (Moser et al. 1989). Furthermore, kinetoplast minicircles constitute roughly 9% of the total organismal DNA; however, the 1,450 base-pair minicircle sequences of *T. cruzi* has only four copies of the 120 base-pair highly conserved region (Moser

et al. 1989). The 120 base-pair minicircle region of *T. cruzi* contains some sequence similarity to the replication origins of minicircle DNA in other trypanosomatids, which reduces PCR specificity. Additionally, the kinetoplastid minicircles are tightly supercoiled, and restriction enzyme cleavage is necessary to ensure accessibility of the target sequences for primer and polymerase binding. Therefore, the abundance and specificity of the 195 base-pair satellite repeat make it an ideal target sequence for PCR detection of *T. cruzi* (Moser et al. 1989).

The Impacts of Predicted Climate Change on Infectious Diseases

Over the last century, global mean surface air temperatures across land and sea have increased beyond any period in the past 40 million years (Intergovernmental Panel on Climate Change (IPCC) 2014). Under scenarios of maximum expected climate change, between 33% and 58% of all species will become extinct by 2050 (Thomas et al. 2004). The emergence of new infectious diseases correlate with socioeconomic, environmental, and ecological factors and are a major public health risk that place an important burden on global economies (Jones et al. 2008).

Several projections estimate climate change will influence the distribution and expansion of tropical diseases, notably vector-borne diseases, throughout temperate regions (Epstein 2000; Lafferty 2009). Examples of concern include schistosomiasis, onchocerciasis, dengue fever, lymphatic filariasis, African and American trypanosomiases, yellow fever, and other mosquito and tick transmitted diseases (Epstein 2000; Lafferty 2009). By 2050, the climate of England will again be suitable for endemic malaria (Department of Health 2002). As climatic temperature and humidity increase, these conditions can influence disease morbidity, most notably the length of transmission season (Hay et al. 2004). For example, malaria transmission can increase to epidemic, hypoendemic, mesoendemic, hyperendemic, and holoendemic levels (Hay et al. 2004; Lafferty 2009). Furthermore, floods and droughts caused by climate change can instigate disease

outbreaks by creating breeding grounds for insects whose desiccated eggs remain viable and hatch in still water (Epstein 2000). Because of observed changes in the distribution and phenology of organisms caused by warming in the 20th century, it is crucial to model how climate change can influence infectious disease ecology within domestic, wildlife, and human populations (Daszak et al. 2000; York et al. 2014).

Modeling the Impacts of Predicted Climate Change on Infectious Diseases

Ecological niche modeling (ENM) is a valuable tool for understanding the geographic ecology of a species. ENM estimates the dimensions of species' ecological niches, which essentially is the space within which a species can maintain populations with immigration (Grinnell 1917; Costa et al. 2002). ENM predicts the fundamental niche (the potential conditions by which an organism can persist) and realized niche (the actual conditions utilized by an organism within their fundamental niche) of a species by relating point occurrence data of a species to environmental factors (Pearson and Dawson 2003; Peterson and Kluza 2005). ENM facilitates the exploration of geographic and ecologic phenomena based on known occurrences of a study species (Peterson 2006; Gurgel-Goncalves et al. 2011; York et al. 2014). Through machine learning, a customized genetic algorithm predicts or confirms the following: high predictive ability of the approach regarding species' distributions, the ability to predict species' potential distributions across scenarios of change on ecologic and evolutionary time scales, the ability to predict the course of species' invasions, the capacity to understand and predict the geographic outcomes of species' interactions, and useful insight into various other aspects of species' distributional ecology (Grinnell 1917; Stockwell and Noble 1992; Peterson and Cohoon 1999; Stockwell 1999; Stockwell and Peters 1999; Peterson et al. 2000, 2001, 2002a, 2002b;

Peterson 2001; Peterson and Vieglais 2001; Costa et al. 2002; Pearson and Dawson 2003; Peterson and Kluza 2005).

Maximum Entropy

Maximum Entropy (MaxEnt) is a specific modeling program that uses species presence only data and environmental conditions to accurately estimate the distribution of a study species (Elith et al. 2011). The role of MaxEnt is to minimize the relative entropy between two probability densities defined in covariate space (Elith et al. 2011). Through predicting the whole geographic range in which a species might occur, the fundamental niche is predicted. This approach can assess the relative importance of specific environmental factors to a species distribution, locate areas of current suitable habitat, and project changes in its distribution over time (Elith et al. 2011).

Understanding the relationship between climate and infectious diseases is increasingly urgent with the predicted effects of climate change on vector-borne and zoonotic diseases (IPCC 2014). Climate change influences the interactions between pathogens and humans, and the interactions between pathogens and vectors and intermediate host species (Gage et al. 2008; Mills et al. 2010). The magnitude and directional changes in climatic variables on the host and vector populations vary locally and are contingent on interactions with physical and biological variables, such as temperature, precipitation, resource competition, and predation (Medone et al. 2015). Triatomines are adapted to a variety of climates and inhabit several environments between tropical and temperate regions.

Potential Climate Change Influence on Trypanosoma cruzi and Triatomine Biology

Geographical distributions, temperature, precipitation, and the biology of important triatomine species are well studied (Grundemann 1947; Lent and Wygodzinksy 1979). Among

these are two leading Latin American species, *Rhodnius prolixus* and *Triatoma infestans*, and five important species in the United States, *T. sanguisuga, T. protracta, T. lecticularia, T. gerstaeckeri,* and *T. indictiva*. *Rhodnius prolixus* range extends from 18° to -3° latitude and -96° to -53° longitude (Medone et al. 2015). *Triatoma infestans* range extends from -11° to -46° latitude and -76° to -51° longitude (Medone et al. 2015). For the five significant *Triatoma* species in the United States, the comvecbined range covers 26 states (CDC 2016 https://www.cdc.gov/parasites/chagas/gen_info/vectors/index.html). *Rhodnius prolixus* inhabits regions with annual mean temperatures from 11°C to 29°C, annual mean precipitation from 250 to 2000 mm, and 0 to 2,600 meters above sea level (Lent and Wygodzinsky 1979). *Triatoma infestans* inhabits regions with annual mean temperatures from -1.6°C to 27.1°C, annual mean precipitation from 0.5 to 2,910 mm, and 0 to 4,100 meters above sea level (Carcavallo et al. 1999; Medone et al. 2015).

An important variable for considering the epidemiologic risk of *T. cruzi* infection is the post feeding-defecation latency time of triatomines, which can be affected by temperature. The most efficient vectors defecate during a blood meal or as soon as they finish blood sucking while still in contact with the host (Zarate et al. 1984). During or shortly after obtaining a blood meal, triatomines separate erythrocytes from plasma and defecate an aqueous mixture, most of which is water and waste products. Each triatomine species varies in their defecation latency time and species with shorter latency time can present a higher risk of infection (Nogueda-Torres et al. 2000; Dorn et al. 2007; Waleckx et al. 2014). The defecation latency time of *R. prolixus* is 9.8 minutes, the defecation latency time of *T. infestans* is 21.3 minutes, and the defecation latency time of *T. lecticularia* is 21.8 minutes (Nogueda-Torres et al. 2000). For *T. protracta,* post feeding defecation times decreased with higher temperatures, with the quickest defecation time

interval of six minutes at 94°F, as opposed to 221 minutes at 66° (Wood 1951). For *T. sanguisuga*, defecation was observed within 20 minutes of feeding (Grundemann 1947).

Temperature is related to host recognition, feeding, egg production and hatching rate, nymphal development time, cessation of molting, and metabolism (Clark 1935; Lazzari and Nunez 1989; Luz et al. 1999; Ferreira et al. 2007; Fresquet and Lazzari 2011). Increasing temperatures directly influences triatomine development and parasite development, and subsequently increases transmission risk.

T. cruzi transmission requires at least 18°C, and higher temperatures increase development in the triatomine gut; however, 38°C is fatal to the parasite (Lambert et al. 2008). Experimentally infected mice with a highly pathogenic strain (Tulahuen) were kept at 25°C and died within 15 days of inoculation. When inoculated mice were kept at 36°, none died within 30 days, 2% died after 60 days, and 8% died after six months post inoculum (Marinkelle and Rodriquez 1968). The authors postulated that high environmental temperature protected the mice against the virulent effects of *T. cruzi*.

Generally, vector-borne parasites cause minimal harm to their vectors, which promotes transmission and ensures the longevity of the parasite (Elliot et al. 2015). Early observations considered *T. cruzi* to be avirulent to triatomines and elicit no parasite-induced changes in triatomine physiology (Juarez 1970; Zeledon et al. 1970; Schaub 1988). Later studies revealed reduced fecundity of infected female triatomines (Botto-Mahan et al. 2008). Elliot et al. (2015) demonstrated altered fecundity and fertility rates of infected *R. prolixus* when exposed to different temperatures. Additionally, *R. prolixus* preferred temperatures ranging 25.0 – 25.4°C, which is also the *in vitro* optimum temperature for *T. cruzi* (Elliot et al. 2015). At this temperature range, *T. cruzi* showed unrestrained growth and increased transmission risk despite

the chance of depriving the infected insect of nutrients, thus eliciting harm towards its vector. The authors observed a direct relationship between higher parasitemia and elevated temperatures. When kept at 30°C, *T. cruzi* increased their numbers approximately 28 times, which doubled their growth rate at 27°C. *T. cruzi* peak growth was observed at or above 30°C and mortality rates were below 5% at 27°C, 15% at 24°C, but increased to 20% at 21°C (Elliot et al. 2015). Elliot et al. (2015) hypothesize low temperatures affect the endocytic process of epimastigotes (Dunn et al. 1980; Figueiredo and Soares 2000).

Infected *R. prolixus* demonstrated delayed molting times with varying temperatures by 6-33 days through resource competition, which favors the survival of *T. cruzi* (Elliot et al. 2015). At 28°C, *T. cruzi* trypomastigotes required one month to colonize the triatomine gut and differentiate into epimastigotes (Schaub 1989). Maturing triatomines only fed after they had molted. Therefore, *T. cruzi* transmission is enhanced via delayed molting and increased consecutive bloodmeal intervals so the parasite can better establish and divide before defecation (Elliot et al. 2015). This behavior can alter the epidemiology of *T. cruzi*, as triatomines express habitat and temperature preference and will likely colonize regions with a favorable climate.

Goals of this Study

T. cruzi is a neglected tropical parasite that is emerging into the United States. Despite three historical reports of *T. cruzi* in Oklahoma wildlife, the endemicity of *T. cruzi* is poorly studied in the state. The overall objective of this project was to improve disease surveillance for *T. cruzi* in Oklahoma and better understand its endemicity by assessing a parasite entry route through a migratory reservoir host, the Mexican free-tailed bat. Chapter two highlights the project's research design, methods, and findings. Chapter three focuses on the potential impacts of global climate change on the current and future potential distribution of *T. cruzi* and five

important *Triatoma* species in Oklahoma and the United States. The knowledge acquired from this project will benefit disease surveillance researchers, wildlife disease ecologists, parasitologists, physicians, and public health officials. This study will contribute to the growing body of knowledge involving *T. cruzi* epidemiology and emergence in the United States.

The research presented in the next two chapters are each formatted as papers for publication in two different journals. Chapter two is formatted and accepted for publication in the Journal of Wildlife Diseases. Chapter three will be submitted to the Centers for Disease Control and Prevention's Journal of Emerging Infectious Diseases.

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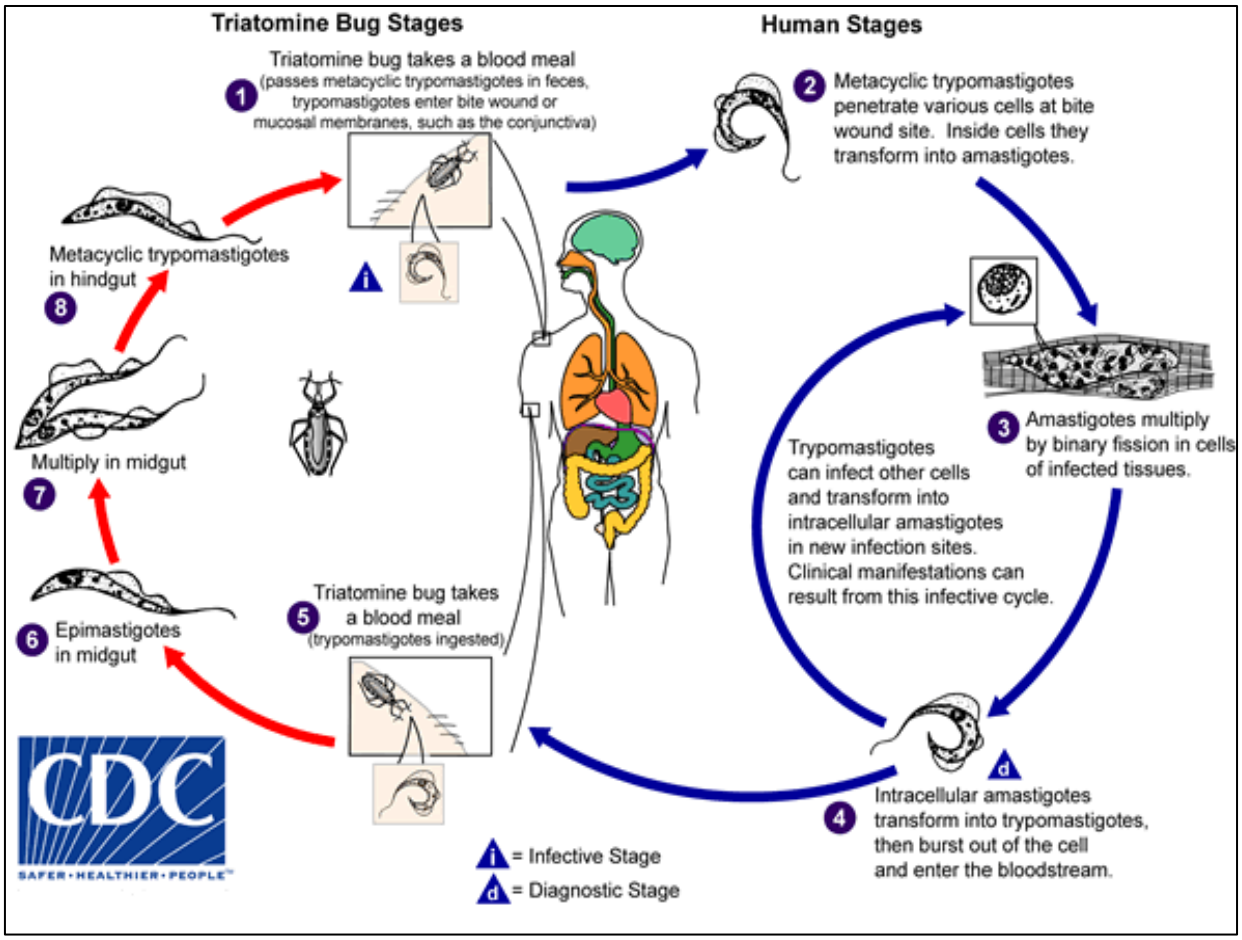

Figure 1. *Trypanosoma cruzi* life cycle illustrated with transmission and maturation in vertebrate (mammal) and invertebrate (triatomine) hosts. Obtained from Centers for Disease Control and Prevention 2015 https://www.cdc.gov/parasites/chagas/biology.html.

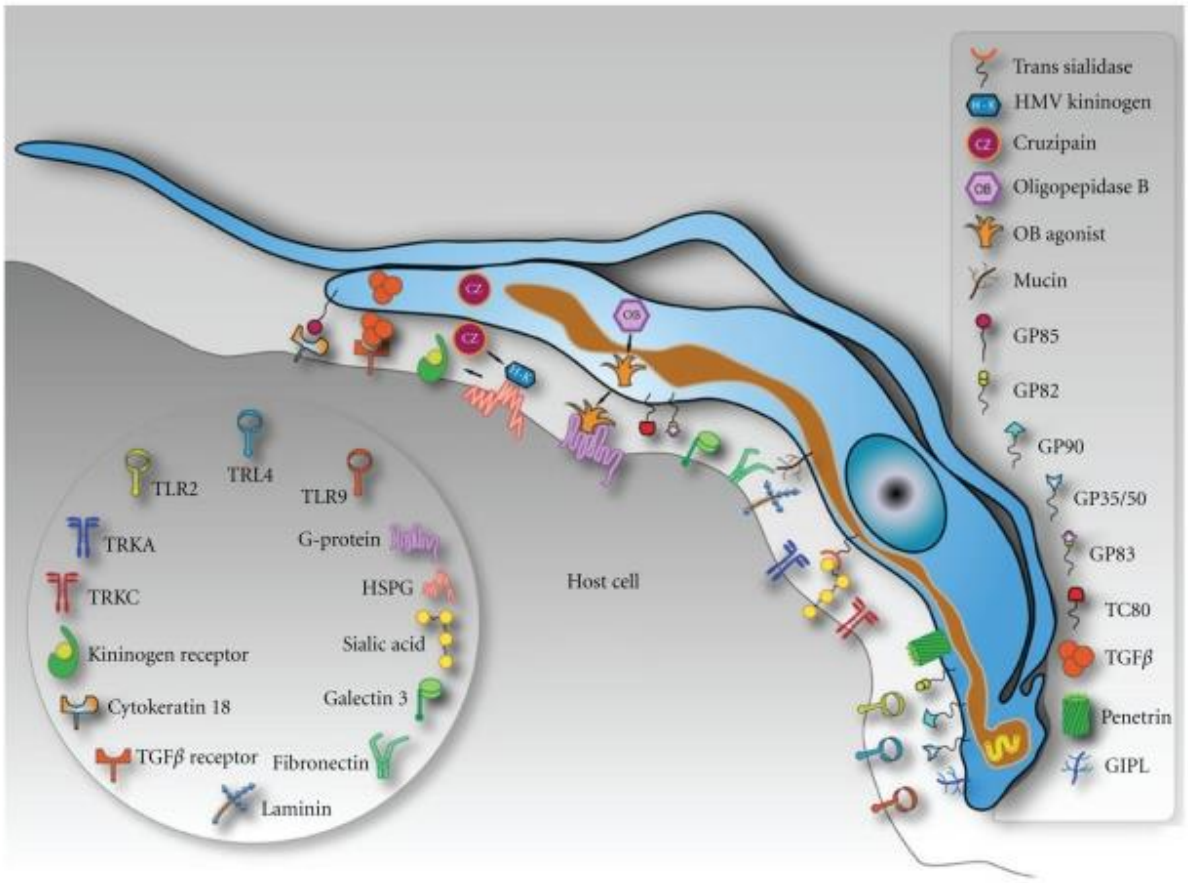

Figure 2. Schematic model summarizing the molecules involved in the parasite-host cell interaction process on the surface of a mammalian host cell and on a trypomastigote of *Trypanosoma cruzi* (obtained from Souza et al. 2010).

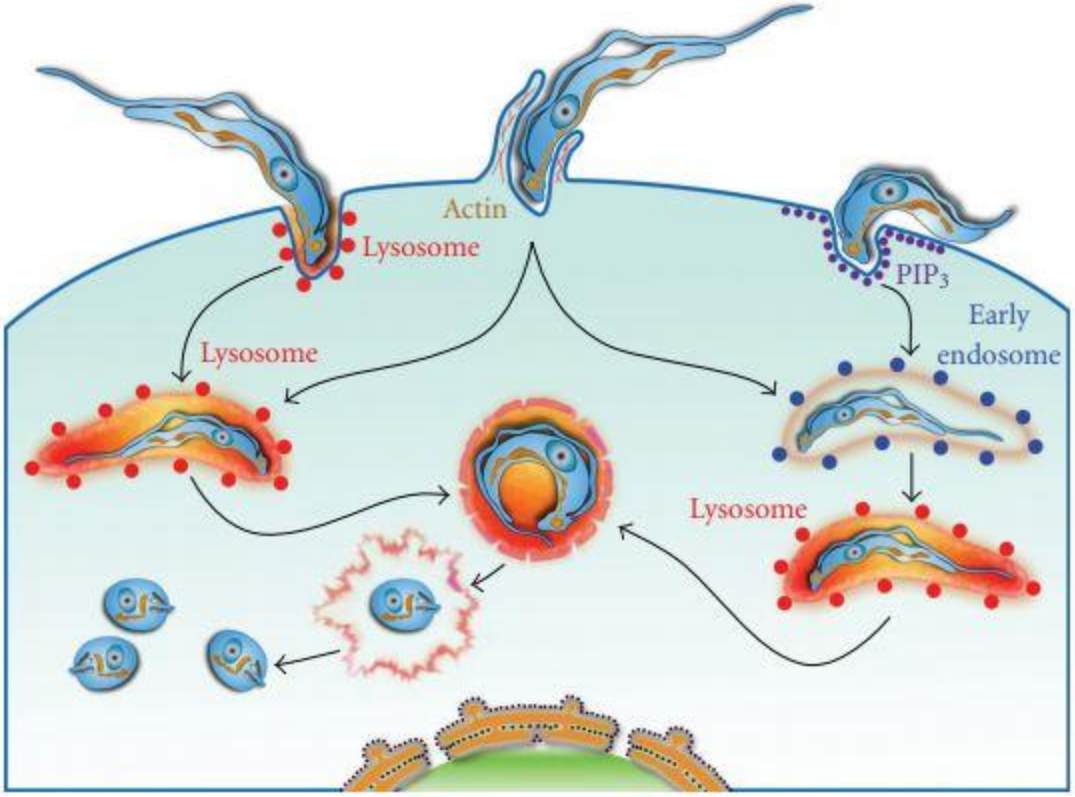
Actin
Lysosome
PIP3
Early
endosome
Lysosome
Lysosome

Figure 3. A model of *Trypanosoma cruzi* intracellular invasion (obtained from Souza et al. 2010). The model represents three mechanisms of *T. cruzi* entry into a mammalian host cell. The first, the lysosome dependent pathway, which is initiated by targeted Ca^{2+} regulated exocytosis of lysosomes within the plasma membrane. The second mechanism is the actin dependent pathway in which the invading trypomastigotes penetrate the host cell via a plasma membrane expansion that promotes the assembly of a parasitophorous vacuole. Endosomes or lysosomes then fuse with the parasitophorous vacuole. The third mechanism involves a lysosome independent pathway, in which the trypomastigote enters a cell via plasma membrane invaginations that then accumulate PIP_3 (a product of class I PI3K activation). The trypomastigote is contained in a vacuole formed from the plasma membrane and attracts early endosome markers (rab5 and EEA1). Lysosome markers are then attracted. The trypomastigote transforms into an amastigote when the parasitophorous vacuole lyses. The amastigote then divides within the cytoplasm (Souza et al. 2010).

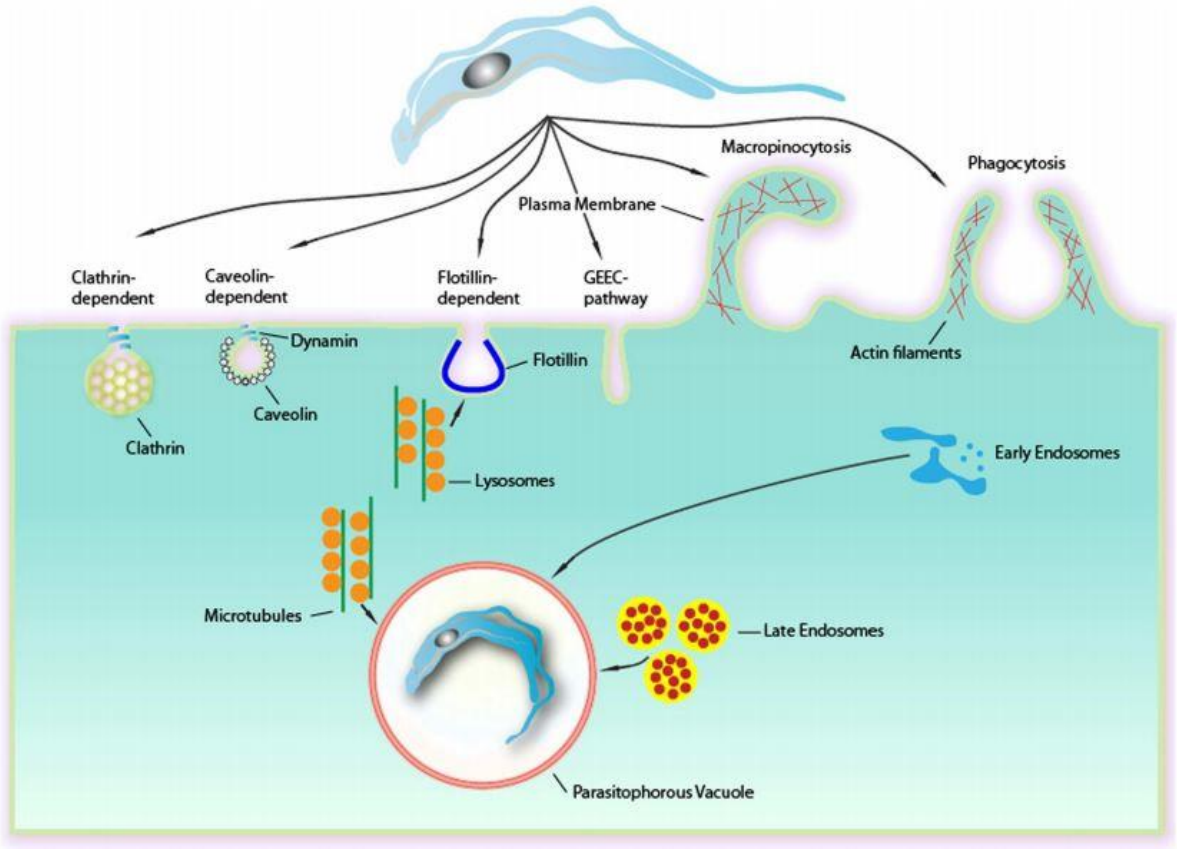

Figure 4. Representation of various endocytic mechanisms involved when *Trypanosoma cruzi* invades a mammalian cell (obtained from Barrias et al. 2013). The formation and maturation of the parasitophorous vacuole relies on the fusion of lysosomes. The fusion of endosomes and lysosomes promotes the maturation of the parasitophorous vacuole through their acidification (Barrias et al. 2013).

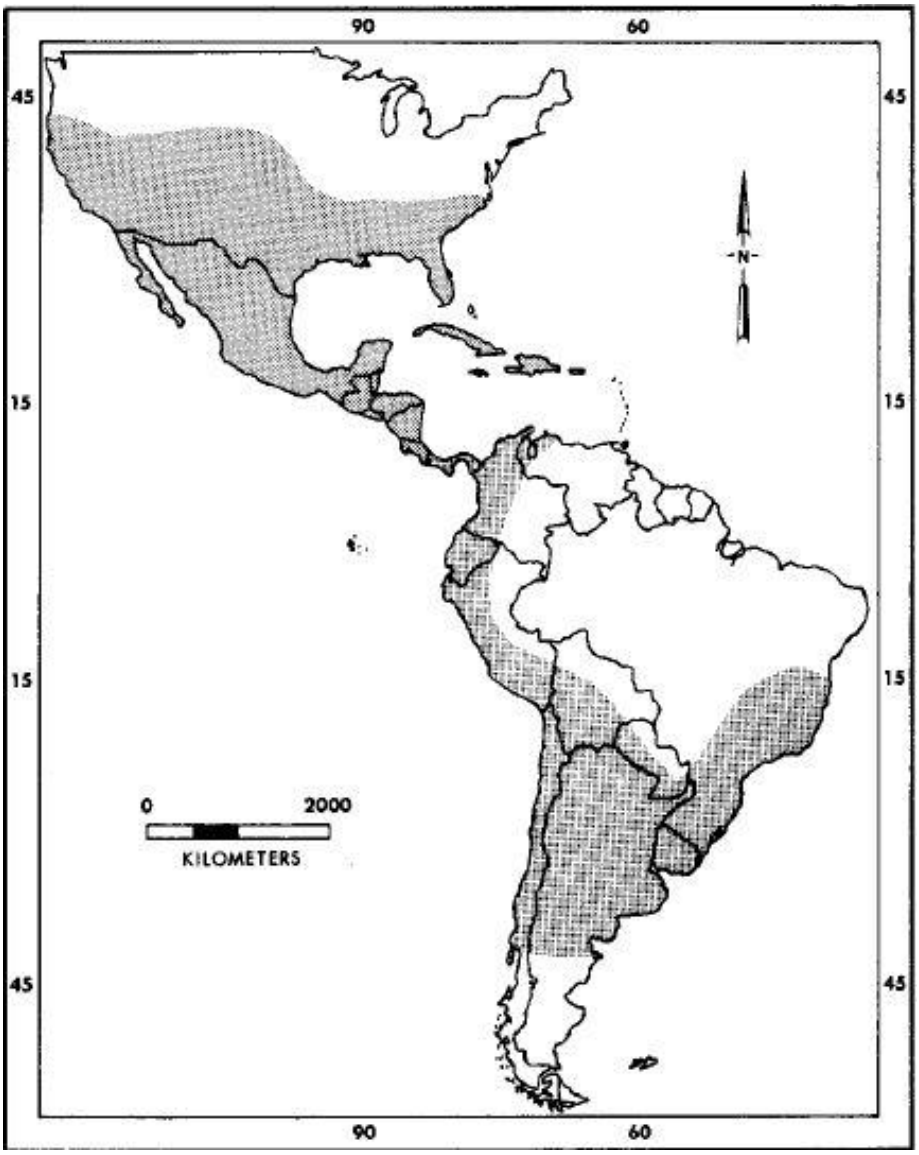

Figure 5. The range of the migratory Mexican free-tailed bat (*Tadarida brasiliensis*; Wilkins 1989). This range spans multiple *Trypanosoma cruzi* endemic foci. During the fall, the population in Oklahoma migrates south through Texas and into Mexico along the Sierra Madre Oriental before returning in the spring to give birth at maternity roosts (Glass 1982).

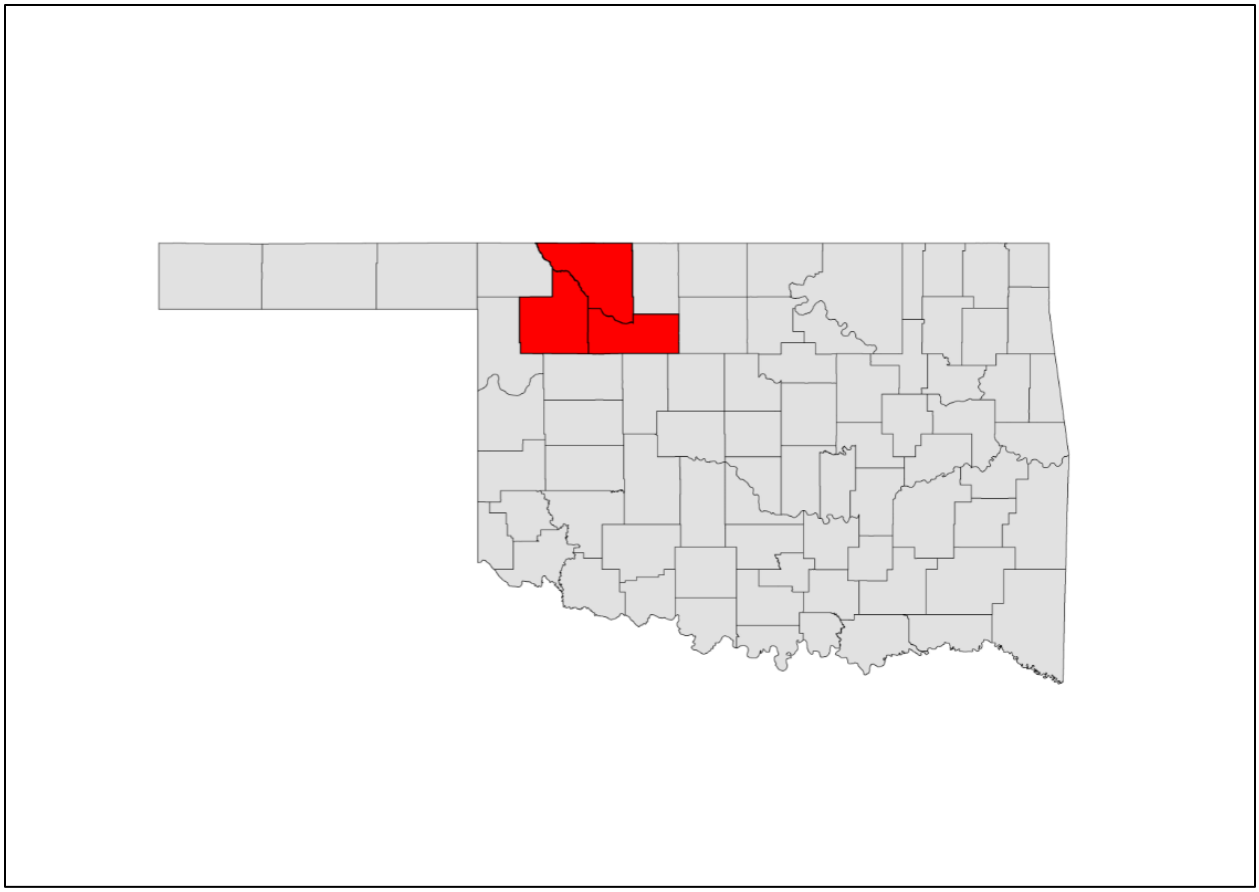

Figure 6. Study area map of northwest Oklahoma, USA illustrating Woodward, Major, and Woods counties in red. Mexican free-tailed bats (*Tadarida brasiliensis*) were sampled at maternity roosts within these three counties during the summer of 2017. The map was created using ArcMap (Environmental Systems Research Institute).

FIRST REPORT OF *TRYPANOSOMA CRUZI* IN A MEXICAN FREE-TAILED BAT (*TADARIDA BRASILIENSIS*) IN OKLAHOMA

ABSTRACT

Trypanosoma cruzi is a vector-borne protozoan parasite that infects seven million individuals in Central and South America and is the etiological agent of Chagas disease. There are increasing reports of endemic transmission within the southern United States. There are three reports of *T. cruzi*, in wild raccoons and dogs, in Oklahoma, but its endemicity in the region is poorly studied. I suspect Mexican free-tailed bats (*Tadarida brasiliensis*) contribute to the endemicity of *T. cruzi* in Oklahoma by their annual migration from Central America to North American maternity roosts. During the summer of 2017, I sampled 361 Mexican free-tailed bats at three maternity roosts in Oklahoma for *T. cruzi*. Wing tissues were collected and *T. cruzi* DNA was extracted, amplified by PCR using the primers TCZ1/TCZ2, and observed by gel electrophoresis. One juvenile Mexican free-tailed bat was positive for *T. cruzi* resulting in a 0.27% prevalence in the 361 sampled bats. This finding is the first reported detection of a wild bat naturally infected with *T. cruzi* in Oklahoma and provides insight on the endemicity of *T. cruzi* in underrepresented endemic areas. The positive sample was sequenced at Eton Biosciences, confirmed as *T. cruzi*, and uploaded to GenBank (MG869732). Future research will focus on monitoring *T. cruzi* prevalence in wild bats and insect vectors to better understand the enzootic emergence of this neglected tropical parasite.

Trypanosoma cruzi is a vector-borne protozoan parasite and the etiological agent of American Trypanosomiasis, commonly known as Chagas disease. Currently, seven million people worldwide are infected with *T. cruzi* and up to 40% will develop Chagas disease, which is

a leading cause of cardiomyopathy in endemic regions (Bocchi et al. 2009). *Trypanosoma cruzi* is known to infect over 400 mammalian species (Hoare 1972) and is transmitted when an infected triatomine (Hemiptera: Reduviidae: Triatominae) feeds on a host and defecates onto host skin or mucous membranes (Rassi et al. 2010). Triatomine vectors range from South America to the central United States, with two species documented statewide in Oklahoma (Lent and Wygodzinsky 1979).

Historically, the United States lacked endemic *T. cruzi*, however, contemporary reports of autochthonous vectorial transmission suggest both endemic and enzootic transmission cycles occur in the southern United States (Dorn et al. 2007). The prevalence of endemic *T. cruzi* in Oklahoma is poorly studied and characterized by three reported canine and raccoon infections (Fox et al. 1986; John and Hoppe 1986; Bradley et al. 2000). Given the presence of migratory Mexican free-tailed bat (MFT; *Tadarida brasiliensis*) maternity roosts in Oklahoma, I hypothesized that the MFT play a potential epidemiological role in the endemicity of *T. cruzi*.

MFT are a migratory bat species that range from Argentina to Ohio (Wilkins 1989). During the spring and summer, Oklahoma is home to three million MFT, who subsequently migrate south along the Sierra Madre Oriental into south-central Mexico for the winter (Figure 1; Glass 1982). MFT are gregarious and aggregate in maternity roosts (Wilkins 1989). MFT are natural reservoirs for various *Trypanosoma* species and potentially support the bat seeding hypothesis, which proposes that migratory bats have a unique epidemiological role in the expansion of *T. cruzi* (Pinto et al. 2011; Hamilton et al. 2012; Hodo et al. 2016).

In the summer of 2017, I sampled 361 MFT from three maternity caves in Oklahoma (Appendix A1). The caves are in Woodward, Woods, and Major counties. While collecting samples, I followed mandatory white-nose syndrome decontamination protocols (U.S. Fish and

Wildlife Service 2016) to reduce the spread of *Pseudogymnoascus destructans* and all study methods were approved by the UCO IACUC (IACUC #17004). I used insect sweep nets to catch emerging MFT at cave mouths and collected tissues from the uropatagium and plagiopatagium using sterile 3-mm biopsy punches. I collected tissues in the vascular patagia to sample both intracellular amastigotes and circulating trypomastigotes, which are found in multiple tissues throughout infected hosts (Rassi et al. 2010). Bats were released on site following sample collection. Following the DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland, USA) protocol, I placed biopsy punches in labeled 1.5 mL microcentrifuge tubes containing 180 µL ATL buffer and 20 µL proteinase K and stored the tubes at room temperature.

To increase sample diversity, I divided collection into monthly trips. I expected *T. cruzi* to be in both adults and juveniles, because *T. cruzi* can be transmitted congenitally (Anez et al. 2009). In May, I sampled 91 pregnant females. In June and July, I sampled 198 lactating females and eight adult males. In August, I sampled 29 female juveniles and 35 male juveniles. Females constitute 86.5% of the sample size because females congregate at maternity roosts and males roost separately. The sharp increase of sampled males during August is attributed to the 1:1 ratio of pup gender (Wilkins 1989). I determined age by transilluminating the wing using a headlight to visualize the epiphyseal fusion of the metacarpal-phalangeal joint.

Trypanosoma cruzi strain Sylvio X10 (American Type Culture Collection, Manassas, Virginia, USA) target DNA was amplified and cloned into a plasmid using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California, USA) and transformed into *Escherichia coli* strain Mach1-T1. I purified plasmid constructs from the transformants using the PureLink HiPure Plasmid Miniprep Kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), to serve

as positive control DNA for PCR. I extracted DNA from wing punches using the DNeasy Blood and Tissue Kit following manufacturer protocols.

I amplified a 195 base pair satellite repeat from *T. cruzi* nuclear DNA (nDNA) via PCR using the primers TCZ1/TCZ2 (Virreira et al. 2003). TCZ1/TCZ2 amplify nDNA from all *T. cruzi* lineages and subspecies (Virreira et al. 2003), but do not amplify other *Trypanosoma* species, including nonpathogenic *T. rangeli*. I followed the protocol of Virreira et al. (2003) for PCR. PCR amplicons were electrophoresed on a 1% agarose gel with TAE buffer in the presence of 0.5 µL/mL of ethidium bromide. Additionally, I ran negative controls for each PCR.

I detected *T. cruzi* DNA in one juvenile MFT, resulting in a 0.27% prevalence in the sampled bats (N = 361). The positive sample and control DNA were Sangar sequenced at Eton Biosciences (San Diego, California, USA) and I aligned the forward and reverse sequences using Sequencher 5.4.6 (GeneCodes, Ann Arbor, Michigan, USA). I entered the aligned sequences into BLAST (NCBI) and confirmed the organismal DNA as *T. cruzi*. I compared closely related sequences (Figure 2) and uploaded the sample to GenBank (MG869732). I did not determine the discrete typing unit (DTU) of the positive sample due to sequencing equipment restraints. Multiple closely related sequences were collected from the Las Palomas Wildlife Management Area in southern Texas, which is along the MFT migratory pathway (Aleman et al. 2017). I suspect a female MFT acquired *T. cruzi* while migrating through this WMA as all published Las Palomas sequences and the Sylvio X10 strain belong to the TcI lineage (Hodo et al. 2016).

Low prevalence in the sampled population is likely due to roost location on the northern boundary of the historic triatomine range and low endemic triatomine populations near these sites (Lent and Wygodzinsky 1979). We were unable to trap and confirm vector prevalence and

infectivity due to sampling restrictions imposed by the risk of anthropogenic *P. destructans* contamination.

I present the first report of a wild bat naturally infected with *T. cruzi* in Oklahoma, the second report of a bat naturally infected in the United States, and the fourth reported animal infection in the state (Hodo et al. 2016). I present the first report of *T. cruzi* detection from bat patagia and a convenient and sensitive methodology for *T. cruzi* disease surveillance that can be applied to a variety of wild mammals in underrepresented and endemic areas.

We suggest that MFT potentially contribute to the endemicity of *T. cruzi* in Oklahoma and might contribute to future enzootic expansion. Despite low prevalence in my sample size, MFT might play a unique role in the epidemiology of *T. cruzi* through their annual migration from classical endemic foci. Although the distribution of triatomines in Oklahoma is poorly studied, *Triatoma lecticularia* and *T. sanguisuga* have been identified statewide, including Oklahoma City (Griffith 1947; Drew and Schaefer 1962). In Texas, up to 63% of sampled triatomines statewide were positive for *T. cruzi* (Curtis-Robles et al. 2015) and there are reports of sylvatic transmission cycles in Texas mammals along the MFT migratory pathway (Kjos et al. 2009). Future research will focus on assessing *T. cruzi* prevalence in wild and domestic Oklahoma mammalian reservoirs, identifying foci of sylvatic and peridomestic transmission, surveillance of classic and potentially novel arthropod vectors, and the impending impact of climate change on vector, MFT, and *T. cruzi* biogeography in Oklahoma.

I thank the numerous research volunteers who assisted in sample collection, the Oklahoma Department of Wildlife Conservation, and the landowners of the maternity roosts. I thank Dr. William Caire for the project structure and mammalogy training; and Dr. James Creecy for verifying our work with Sequencher, providing molecular analyses advice, and manuscript

review. I thank Sarah Vrla for her editorial comments. Support for this research was provided by the University of Central Oklahoma, Student Transformative Learning Record; Research, Creative, and Scholarly Activities; the UCO Center for Wildlife Forensic Science and Conservation Studies; the W. Roger Webb Forensic Science Institute; and the College of Mathematics and Sciences.

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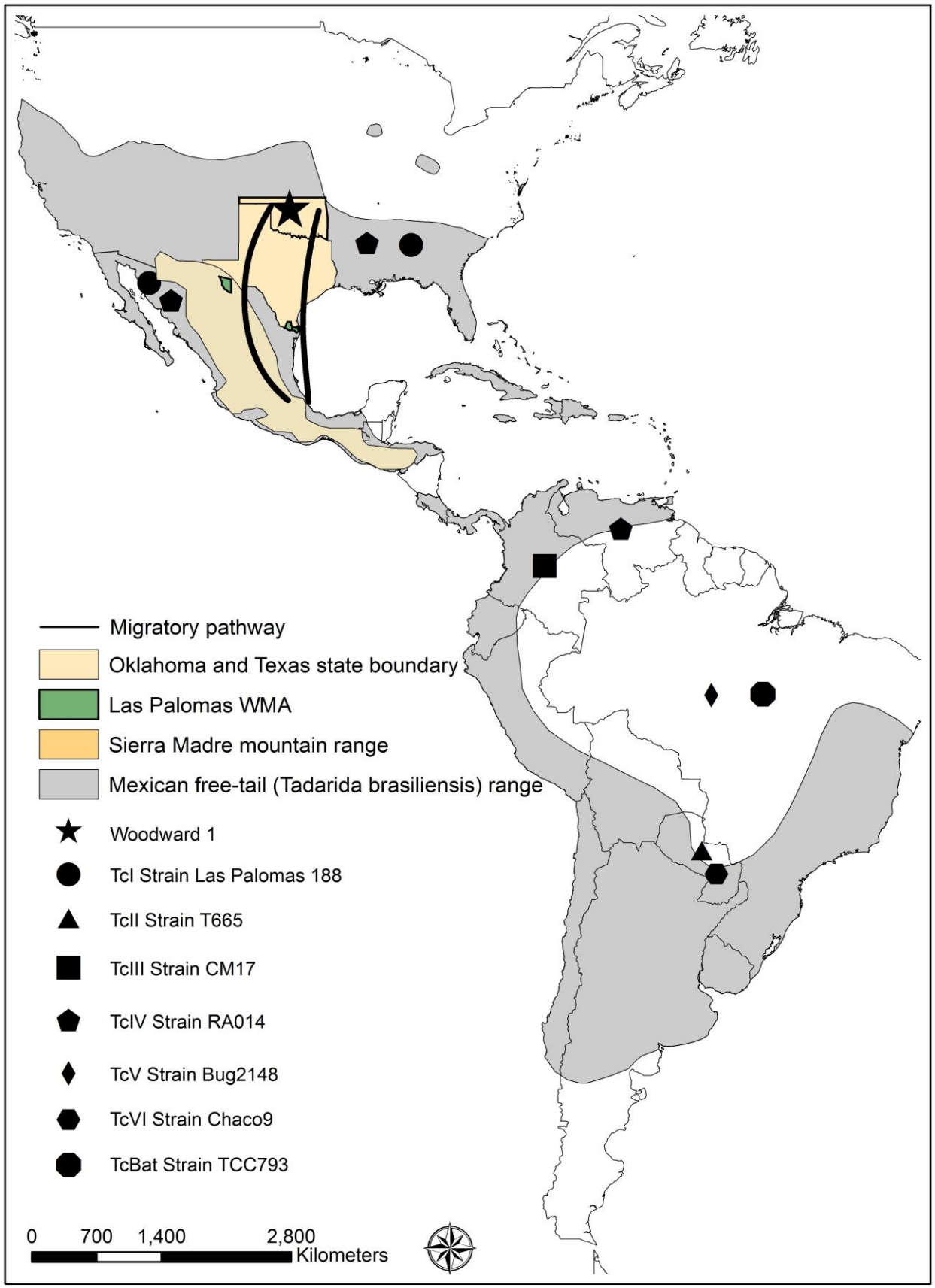
Migratory pathway
Oklahoma and Texas state boundary
Las Palomas WMA
Sierra Madre mountain range
Mexican free-tail (Tadarida brasiliensis) range
Woodward 1
TcI Strain Las Palomas 188
TcII Strain T665
TcIII Strain CM17
TcIV Strain RA014
TcV Strain Bug2148
TcVI Strain Chaco9
TcBat Strain TCC793
0 700 1,400 2,800
Kilometers

FIGURE 1. The range of the migratory Mexican free-tailed bat (*Tadarida brasiliensis*; World Health Organization and International Union for Conservation of Nature and Natural Resources 2015 http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T21314A22121621.en). This range spans multiple *Trypanosoma cruzi* endemic foci. During the fall, the population in Oklahoma migrates south through Texas and into Mexico along the Sierra Madre Oriental before returning in the spring to give birth at maternity roosts (Glass 1982). The positive sample, sequence Woodward 1 matched closely to multiple Las Palomas Wildlife Management Area sequences on GenBank, which is along the migratory pathway. The representative *T. cruzi* lineages we used in the phylogenetic analysis are illustrated. Sampling occurred at three maternity roosts in Oklahoma during the summer of 2017.

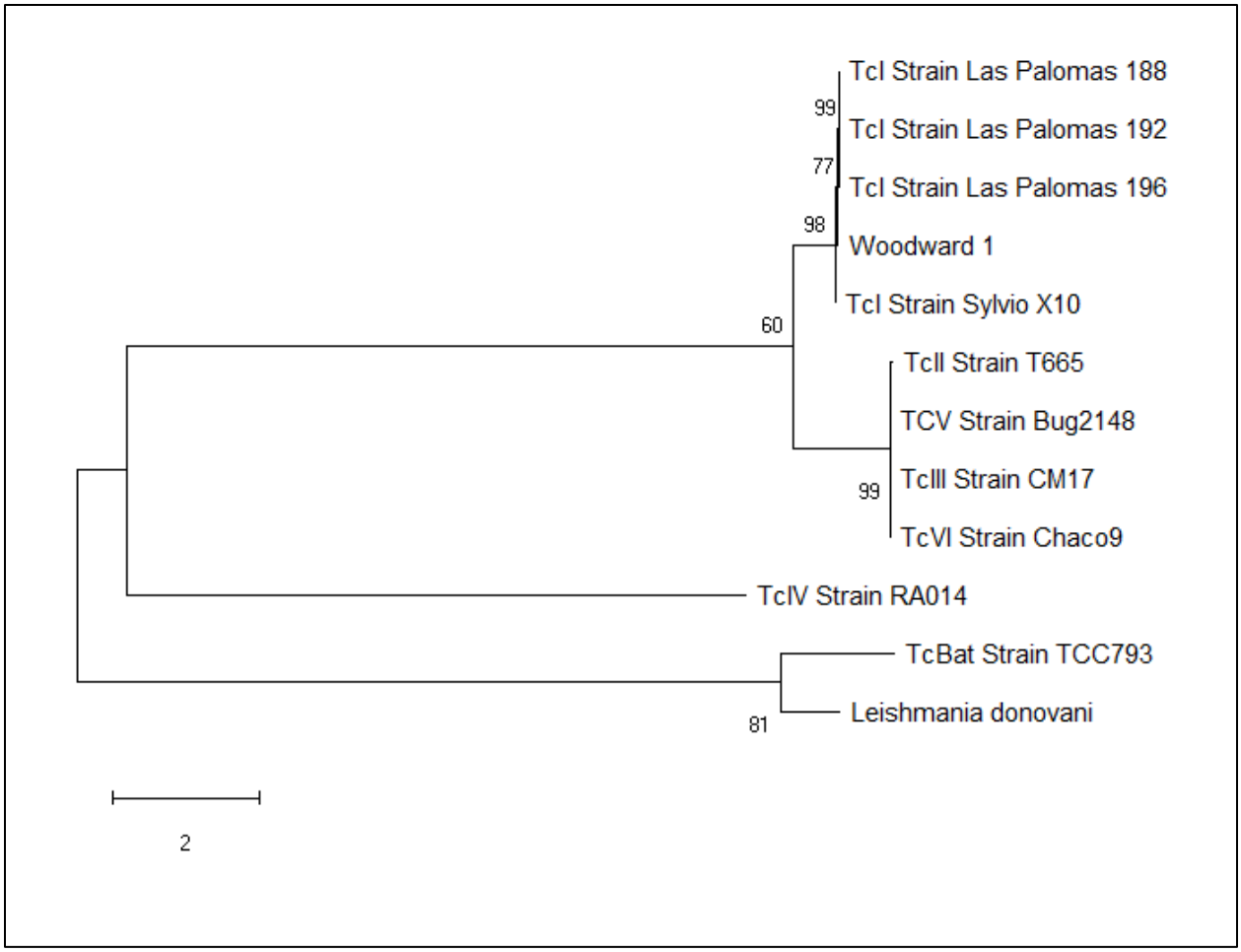

FIGURE 2. Phylogenetic analysis of the positive sample, Woodward 1, with closely related sequences. Woodward 1 is closely related to multiple Las Palomas sequences, which were collected from the Las Palomas Wildlife Management Area in southern Texas, USA (Aleman et al. 2017). All six *Trypanosoma cruzi* discrete typing units are represented, including the newly discovered TcBat strain. *Leishmania donovani*, a sympatric trypanosomatid, is the outgroup. All sequences were acquired from GenBank. The evolutionary history was inferred using the Maximum Likelihood method based on the Jukes-Cantor model with 1000 bootstrap replicates. Evolutionary analyses were performed using Mega7 (Kumar et al. 2015).

PROJECTED EXPANSION IN CLIMATIC SUITABILITY FOR *TRYPANOSOMA CRUZI*, THE ETIOLOGICAL AGENT OF CHAGAS DISEASE, AND FIVE WIDESPREAD *TRIATOMA* SPECIES BY 2070

ABSTRACT

The vector-borne hemoflagellate parasite *Trypanosoma cruzi* infects seven million individuals globally and causes chronic cardiomyopathy and gastrointestinal diseases. Historically, *T. cruzi* was endemic to Central and South America but is now found throughout the southern United States and across 43 countries globally. Several projections estimate climate change will alter the distribution and facilitate the expansion of tropical diseases, notably vector-borne diseases, throughout temperate regions. Given that *T. cruzi* is a neglected tropical parasite that persists in temperate regions, such as Oklahoma, it is crucial for disease surveillance efforts to detail current and future regions that present favorable climatic conditions for *T. cruzi* and vector establishment. I used the program MaxEnt to develop an ecological niche model for *T. cruzi* and five widespread *Triatoma* vectors based on 546 published localities within the United States and 19 bioclimatic variables. I modeled regions of current potential *T. cruzi* and *Triatoma* distribution and then regions projected to have future potential suitable climatic conditions under a Representative Concentration Pathway (RCP 8.5) scenario by 2070. Regions with potential suitable climatic conditions for *T. cruzi, T. lecticularia, T. protracta, T. indictiva,* and *T. sanguisuga* are predicted to increase within the United States and Oklahoma by 2070. Regions with potential suitable climatic conditions for *T. gerstaeckeri* are predicted to increase within the United States but not into Oklahoma by 2070. Our findings agree with previous literature and

confirm that climate change will influence the expansion of *T. cruzi* and important *Triatoma* vectors in Oklahoma and the United States.

INTRODUCTION

Trypanosoma cruzi is a vector-borne hemoflagellate parasite and the etiological agent of American Trypanosomiasis, also known as Chagas disease. Currently, *T. cruzi* infects seven million people across 43 countries and up to 40% will develop Chagas disease, which causes cardiomyopathy, tissue fibrosis, lethargy, gastrointestinal diseases such as megaesophagus and megacolon, and 10,000 deaths annually (*1-5*). *Trypanosoma cruzi* is transmitted when infected hematophagous triatomines (Hemiptera: Reduviidae: Triatominae) feed on a host and defecate onto the host skin or mucous membranes, thereby allowing the parasite to enter the host via the bite wound (*4*). *Trypanosoma cruzi* is known to infect over 400 mammalian species and is prevalent within wildlife populations in endemic regions where triatomine vectors occur (*6-9*).

Current trends suggest global climate change will result in an expansion of tropical diseases, notably vector-borne diseases, throughout temperate regions (*10-11*). Examples of concern include schistosomiasis, onchocerciasis, dengue fever, lymphatic filariasis, African and American trypanosomiases, yellow fever, and other mosquito and tick-transmitted diseases of humans (*10-11*). By 2050, the climate of England will again be suitable for endemic malaria (*12*). As climatic temperature and humidity increase, conditions can influence disease morbidity, most notably the length of transmission (*13*). For example, malaria transmission can increase to epidemic, hypoendemic, mesoendemic, hyperendemic, and holoendemic levels (*11, 13*). Furthermore, floods and droughts caused by climate change can instigate disease outbreaks by creating breeding grounds for insects whose desiccated eggs remain viable and hatch in still water (*10*).

Over the last 100 years, global mean surface air temperatures over land and oceans have increased beyond any period in the past 40 million years (*14*). One report modeled species extinction and estimated between 33% and 58% of all species will become extinct by 2050 under scenarios of maximum expected climate change (*15*). Additionally, projections estimate climate change will influence the distribution and expansion of tropical diseases, notably vector-borne diseases, throughout temperate regions (*10-11*). Because of the effects of observed changes in the distribution and phenology of organisms caused by warming in the 20th century, it is important to model how climate change may influence infectious disease ecology within domestic, wildlife, and human populations (*16-17*).

Ecological niche modeling (ENM) is a valuable tool for understanding the geographic ecology of a species. ENM estimates the dimensions of species' ecological niches, which is the space within which a species can maintain populations with immigration (*18-19*). ENM predicts the fundamental and realized niches of species by relating point occurrence data of species to environmental factors (*20-21*). Through machine learning, a customized genetic algorithm predicts and confirms the following: high predictive ability of the approach regarding species' distributions, the ability to predict species' potential distributions across scenarios of change on ecologic and evolutionary time scales, the ability to predict the course of species' invasions, the capacity to understand and predict the geographic outcomes of species' interactions, and useful insight into various other aspects of species' distributional ecology (*18-30*).

ENM is an essential tool for understanding the geographic dimensions of the risk of transmission of *T. cruzi*. ENM facilitates the exploration of geographic and ecologic phenomena based on known occurrences of the study species (*17, 31-32*). ENM is used to better understand the epidemiology of *T. cruzi* through niche characterization of triatomines, and relationships

between vector and reservoir distributions (*19*, *33*). Studies of the geographic distribution of *T. cruzi* and widespread *Triatoma* vectors are crucial for understanding the epidemiologic aspects of *T. cruzi* transmission and must be taken into consideration when focusing control efforts and disease surveillance in underrepresented areas (*32*).

Maximum Entropy (MaxEnt) modeling uses species presence-only data and environmental conditions to estimate the distribution of a species (*33*). The basis of MaxEnt is to minimize the relative entropy between two probability densities defined in covariate space (*33*). By predicting the entire geographic range in which a species might occur, the realized niche does not limit the fundamental niche. This approach can be used to assess the relative importance of specific environmental factors to a species distribution, locate areas of current suitable habitat, and project changes in its distribution over time (*33*).

Recently, increasing reports of autochthonous vectorial transmission suggest *T. cruzi* is endemic in the United States and enzootic transmission cycles are more prevalent than expected (*5*, *34-43*). Currently, there are 29 states with reports of *T. cruzi* and triatomine vectors (*5*). There are four reports of *T. cruzi* in Oklahoma wildlife, but its endemicity within the state is underrepresented when compared to other southern states (*34-36*, *43*).

The World Health Organization instigated a vector control and eradication program in Latin America which has substantially decreased transmission in rural regions of Latin American and reduced disease incidence by 94% in the Southern Cone countries (*44*). Decades of successful vector eradication campaigns in Latin America, along with regional programs focused on reducing vector infestation within human dwellings and blood screening, decreased the total prevalence of Chagas disease from >16 million to 8 million people (*4*, *45-46*).

Since 2007, *T. cruzi* control efforts in Latin America were unified to combat the globalization of Chagas disease, which addresses the immigration of infected individuals into non-endemic countries and the potential for non-vectorial transmission routes (*3, 5*). Despite the successful eradication programs and the effort to minimize globalization, *T. cruzi* infections have spread globally through human immigration (*5, 45, 47*). The United States has an estimated 300,000 infected individuals, most of which are immigrants from areas endemic to *T. cruzi* (*5*). With the emergence of *T. cruzi* in the United States*;* the widespread distribution of vectors within the United States; and projected effects of climate change on parasite, vector, and reservoir distribution; there is a need to determine the potential current and future distributions of these organisms to better understand their influence on the epidemiology of *T. cruzi* in the United States.

METHODS

I used Maxent and the 'ENMeval' package in R (*48*) to model the current and projected distribution of *T. cruzi* as well as five widespread potential vectors: *Triatoma gerstaeckeri*, *T. indictiva*, *T. lecticularia*, *T. protracta*, and *T. sanguisuga* (*49-51*). I collected documented occurrences of these six species from published records and incorporated records that met one or more of the following criteria: 1) documentation of the parasite in accepted endemic areas; 2) multiple cases of human infection (three or more) within an area; and 3) reports of the parasite found in intermediate or definitive hosts. I included 546 published location data points of *T. cruzi* and the five *Triatoma* species in this study (Appendices A2 and A3), and downloaded elevation and 19 bioclimatic variables from WorldClim (Table 1; *52-53*; http://www.worldclim.org/) at a resolution of 10 arc minutes (400 km^2). I avoided model overfitting using a regularization approach which introduced a penalty for an increase in model complexity (*50,54*), and the small

sample corrected variant of Akaike's information criterion (AICc) scores was used to evaluate the regularization of models (*55*). Future climate conditions for 2070 using the IPCC 5 data from WorldClim (*52*) were used to project the potential future distribution of the six species of interest at 10 arc minutes using the model that best predicted the current distribution of each species. Projected distributions were computed based on the IPCC scenario RCP 8.5 (emissions increase throughout the 21st century) using the ACCESS1.3 general circulation models.

RESULTS

I obtained location data from 215 published records for *T. cruzi* (Appendix A2). Current potential suitable climatic conditions for *T. cruzi* include nearly half of the United States and nearly all of Oklahoma. Areas with suitable climatic conditions for *T. cruzi* are predicted to increase in the United States and Oklahoma by 2070 under the RCP 8.5 scenario (Fig. 1; Table 2). I obtained location data from 70 published records for *T. gerstaeckeri* (Appendix A3). Areas with suitable climatic conditions for *T. gerstaeckeri* are predicted to increase in the United States by 2070 under the RCP 8.5 scenario (Fig. 2; Table 2). At a lower resolution, the potential distribution includes areas of Oklahoma in 2070; however, with a finer resolution, current and future potential distributions do not include areas of Oklahoma (Fig. 2). I obtained location data from 12 published records for *T. indictiva* (Appendix A3). Areas with suitable climatic conditions for *T. indictiva* are predicted to drastically increase in the central and northern United States by 2070 under the RCP 8.5 scenario (Fig. 3). The potential distribution of *T. indictiva* is predicted to increase in Oklahoma by 2070 under the RCP 8.5 scenario (Fig. 3; Table 2). I obtained location data from 51 published records for *T. lecticularia* (Appendix A3). Current areas with potential suitable climatic conditions are found throughout the central and eastern United States (Fig. 4). Areas with suitable climatic conditions for *T. lecticularia* are predicted to

increase in the United States and Oklahoma by 2070 under the RCP 8.5 scenario (Fig. 4; Table 2). I obtained location data from 69 published records for *T. protracta* (Appendix A3). Current areas with potential suitable climatic conditions are found throughout the central and western United States (Fig. 5). Areas with suitable climatic conditions for *T. protracta* are predicted to increase in the United States and Oklahoma by 2070 under the RCP 8.5 scenario (Fig. 5; Table 2). Using a finer resolution for Oklahoma, the potential distribution of *T. protracta* increases into the Oklahoma panhandle by 2070 (Fig. 5). I obtained location data from 130 published records for *T. sanguisuga* (Appendix A3). Current areas with potential suitable climatic conditions are found throughout the central and eastern United States (Fig. 6). Areas with suitable climatic conditions for *T. sanguisuga* are predicted to dramatically increase in the United States and Oklahoma by 2070 under the RCP 8.5 scenario (Fig. 6; Table 2). Using a finer resolution for Oklahoma, the potential distribution of *T. sanguisuga* increases statewide by 2070 (Fig. 6). The bioclimatic variables that contributed the most to predicting the potential distribution of *T. cruzi* and the *Triatoma* vectors were annual mean temperature, mean diurnal range, annual precipitation, max temperature of the warmest month, and precipitation of the warmest quarter.

DISCUSSION

Infectious diseases are predicted to emerge in novel foci because of the potential implications of global climate change on the pathogen and vector/host biogeography (*10-11*). The influence of climate change will manifest as an increase of disease outbreaks in current regions, and expanded transmission risk and disease emergence to novel regions (*10, 56-58*). Vector-borne diseases will see marked increases in pathogen and vector distribution. By 2085, total land area favorable for Dengue fever transmission will place up to 60% of the global population at risk for infection (*59*). The sandfly, a prominent vector of *Leishmania* parasites, is

predicted to expand northward into the United States and can increase transmission risk in novel foci (*60*). With the future expansion of pathogens into novel foci, there can be a shift or decline in habitat suitability in current foci, leading to a decline of infections in current foci (*11, 17, 61*).

Elevating temperature can directly increase the potential for vector-borne diseases and pathogens to increase in disease morbidity (*62-64*). For instance, rises in temperature can increase the development time for *Plasmodium*, *T. cruzi*, and schistosome cercaria (*63, 65-67*). Consequently, elevating temperature may affect the pathogen transmission potential and pathogen mortality might increase.

Under the hypothesized IPCC climatic scenarios, our model predicts an overall increase in habitat suitability for *T. cruzi* in the United States, which favors an increase in potential distribution by 2070. The vectors *T. gerstaeckeri, T. indictiva, T. lecticularia, T. protracta,* and *T. sanguisuga* also express this trend, which supports previous literature that vector-borne diseases will spread into temperate regions through increases in suitable vector habitat (*10-11*).

Historically, *T. cruzi* and *Triatoma* vectors have plagued humans and animal reservoirs in Central and South America, where climate favored parasite transmission and vector biology. Recent molecular evidence suggests that *T. cruzi* evolved from a bat trypanosome in South America approximately 6.5-8.5 million years ago (*68-70*). Shortly after trypanosome-infected bats colonized South America 7-10 million years ago, South American humans became infected with *T. cruzi* (*71*). The earliest detected human case comes from a 9000-year-old Chinchorro mummy identified via PCR amplification of kinetoplastid DNA sequences (*72*). *Trypanosoma cruzi* infected up to 41% of the Chinchorro population located in the Atacama Desert, and this region is where Chagas disease likely originated (*72-73*). After the Chinchorro population settled and farmed in regions where sylvatic *T. cruzi* cycles occurred, a domestic transmission cycle

emerged (*72-75*). The ability of different triatomine vectors, particularly *T. infestans*, to quickly adapt to human dwellings facilitated the domestic *T. cruzi* transmission cycle (*76*).

Temperature preference can influence the transmission dynamics and epidemiology of *T. cruzi* and competent vectors. Increases in temperature directly increase insect metabolism (*77*). In one study, mice were experimentally inoculated with virulent *T. cruzi* strains and subjected to different temperatures (*78*). When the mice were kept at 10^{O}C, observed parasitemia became severe after nine days, and all the mice died between the 21st and 26th days (*78*). When the mice were kept at 35^{O}C, trypanosomes were undetectable in the blood and from sections of the heart (*78*). When the mice were at 26^{O}C, the mice developed a chronic infection (*78*). In another study, mice were experimentally infected with a virulent strain, and all mice maintained at 25 ± 2^{O}C died 9-15 days post inoculation (*65*). These findings suggest a high environmental temperature protected the mice against the virulent effects of *T. cruzi*.

One group of authors examined the influence of temperature on the development of *T. cruzi* while in *Rhodnius prolixus*, which is a common South American vector (*67*). The authors hypothesized that the temperature preference of *R. prolixus* also is an optimum temperature range of *T. cruzi*, which is 25.0-25.4^{O}C (*79*). At this temperature range, *T. cruzi* has a high *in vitro* growth rate and expresses unrestrained growth which increases the transmission risk. The authors noted a direct relationship between *T. cruzi* parasitemia levels and temperature (*67*). When kept at 30^{O}C, *T. cruzi* increased its numbers by 28 times, which doubled its growth rate from 27^{O}C (*67*). Lower temperatures affect the endocytic processes of *T. cruzi* epimastigotes and increase *T. cruzi* mortality (*80*). At 28^{O}C, *T. cruzi* takes one month to colonize the triatomine intestinal tract, reach the rectum, and differentiate into metacyclic trypomastigotes (*81-82*).

Trypanosoma cruzi infected *R. prolixus* instar molts were delayed by more than 10 days per instar stage (*67, 83-84*). Because triatomines only feed after they have molted, it benefits *T. cruzi* to delay their molt and subsequent bloodmeal until *T. cruzi* has colonized, replicated, and is ready to be transmitted, which favors *T. cruzi* transmission potential (*67*).

Although the potential distribution and habitat suitability may be favorable for disease transmission in the United States, many factors inhibit *T. cruzi* from maintaining a high prevalence within humans in the United States. These include the lack of suitable domestic dwellings for local triatomine vectors to colonize, triatomine expressed zoophilicity, varying and/or delayed triatomine post-feeding to defecation time, low virulence of some indigenous *T. cruzi* strains, historic temperate climate, and the possibility of misdiagnosis (*83, 85-87*).

The CDC recommends that physicians, veterinarians, and public health officials should implement blood screenings for patients that exhibit acute symptoms with a history of visiting areas where triatomines have colonized and might have transmitted *T. cruzi*. For chronic cases, PCR and two serology tests should be performed for the diagnostic confirmation. Additionally, I recommend patient history and location should be collected, and those areas should be investigated for triatomines.

I present a potential range expansion for *T. cruzi* and five important *Triatoma* species. For this study, I modeled habitat suitability based on 19 bioclimatic variables. Future studies should compare the infection rates of important vectors, consider the post-feeding defecation times of each vector, and consider human dwellings and peridomestic animals for precise areas of high-risk transmission potential, which are likely areas of poor housing where vectors can readily colonize. Lastly, I urge physicians, veterinarians, public health officials, and researchers to increase disease surveillance for *T. cruzi* and triatomine vectors to better understand the

current and future epidemiology of *T. cruzi*, triatomine vectors, and reservoir hosts in the United States.

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Figure 1. The current potential distribution of *Trypanosoma cruzi* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we predict an increase in future potential suitable climatic conditions statewide. Maps were generated using MaxEnt.

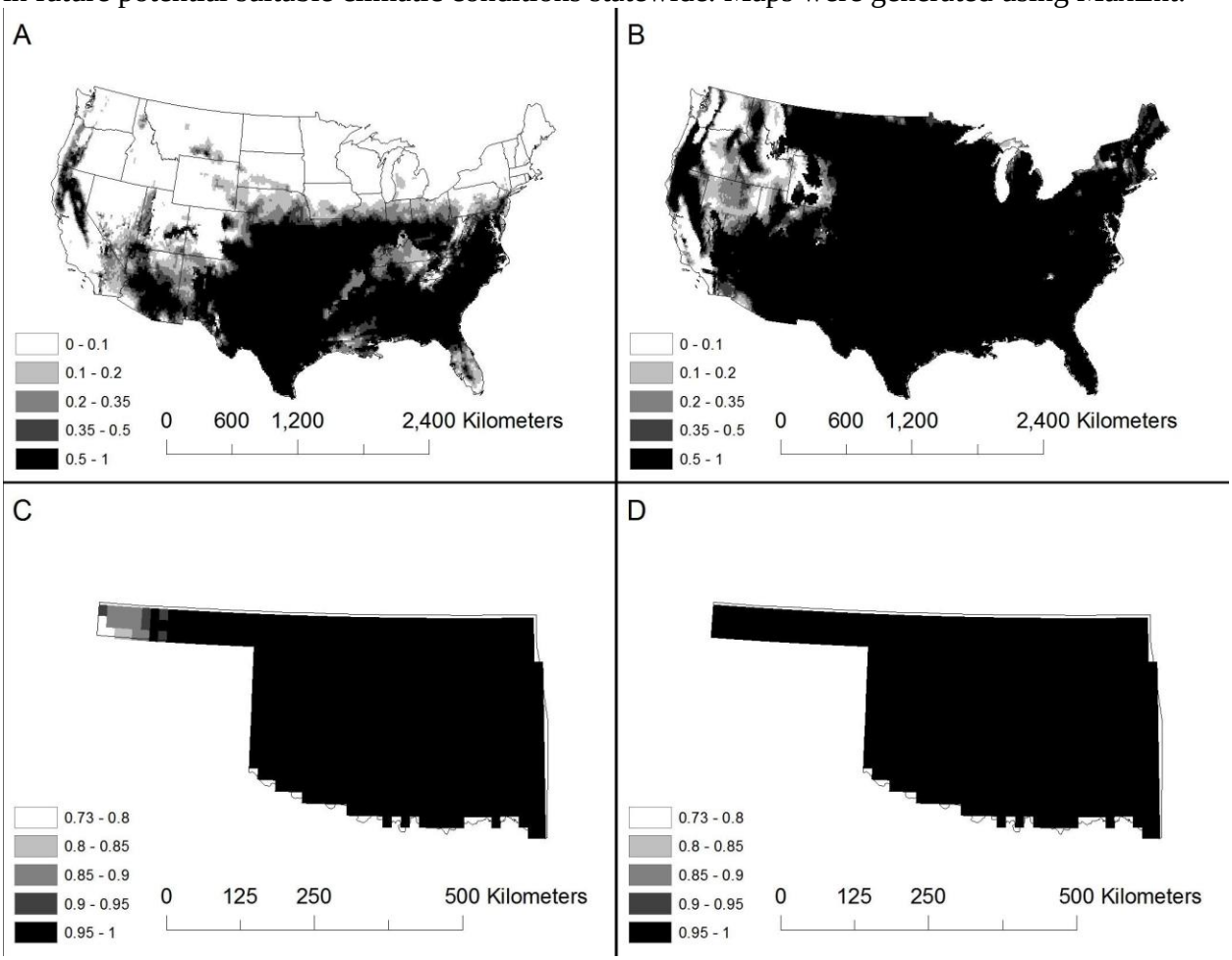

Figure 2. The current potential distribution of *Triatoma gerstaeckeri* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we do not predict an increase in future potential suitable climatic conditions by 2070 for this species. We believe this is due to the historic arid environmental conditions preferred by *T. gerstaeckeri*. Maps were generated using MaxEnt.

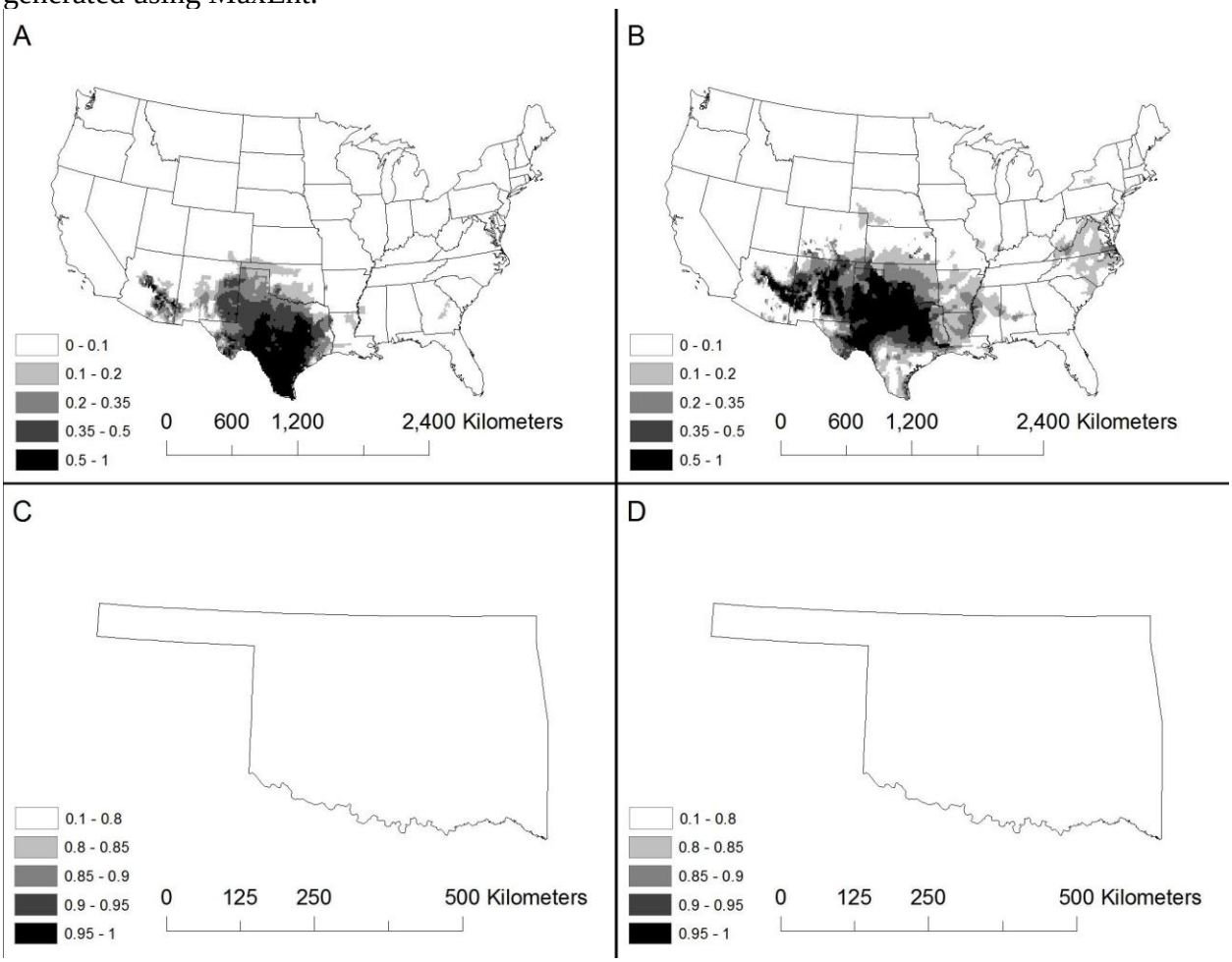

Figure 3. The current potential distribution of *Triatoma indictiva* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we predict an increase in future potential suitable climatic conditions for the majority of the state. Maps were generated using MaxEnt.

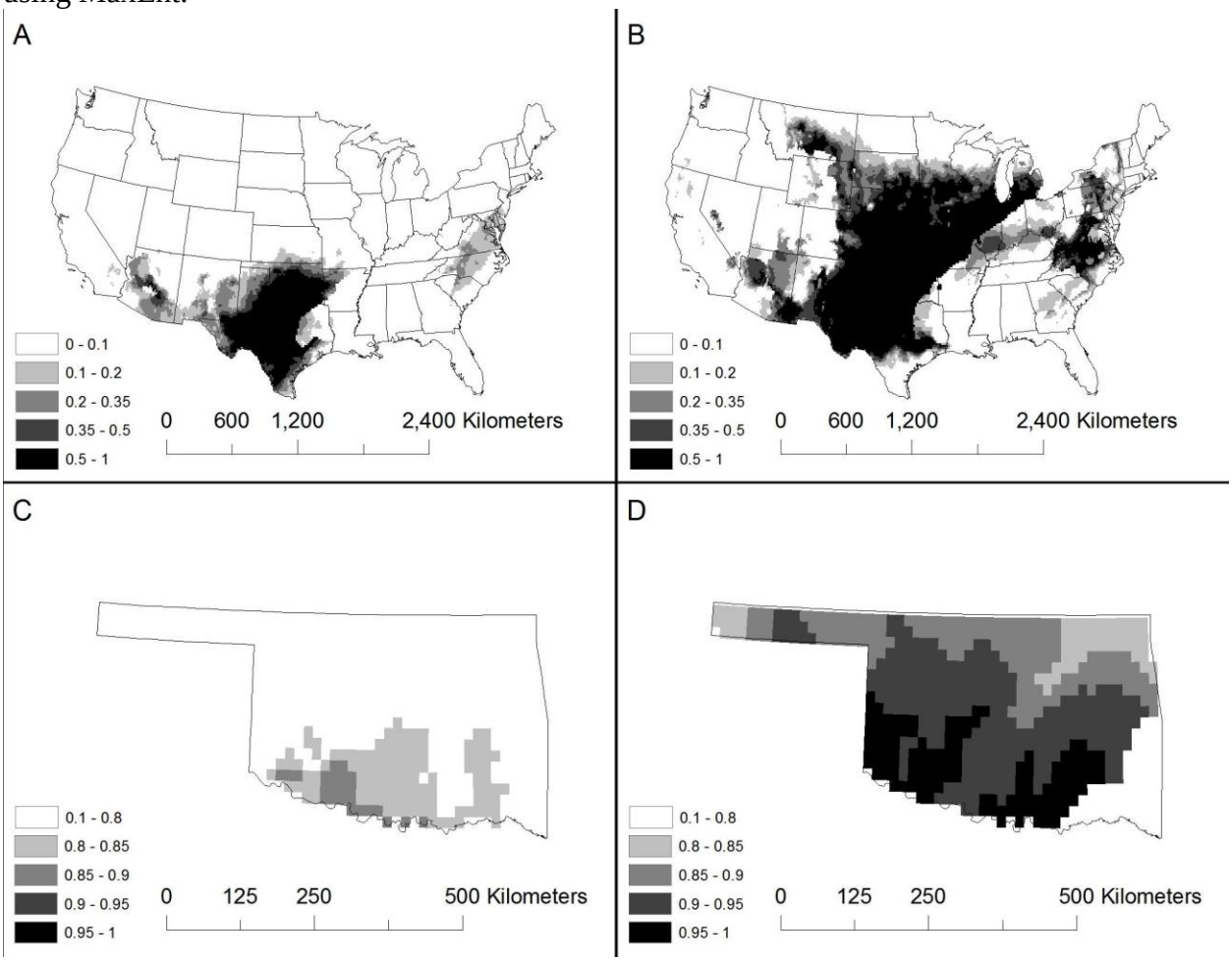

Figure 4. The current potential distribution of *Triatoma lecticularia* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we predict an increase in future potential suitable climatic conditions for the majority of the state. Maps were generated using MaxEnt.

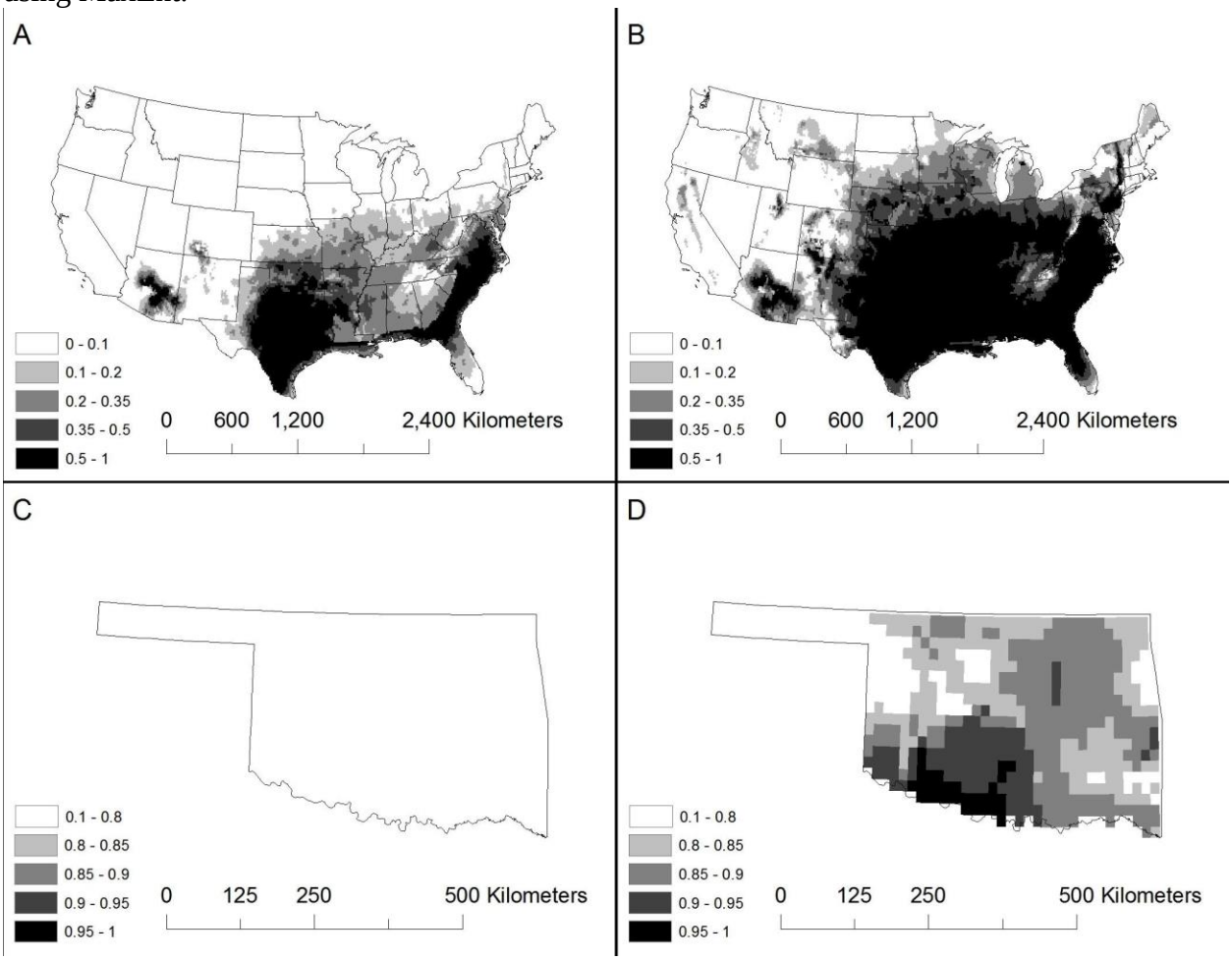

Figure 5. The current potential distribution of *Triatoma protracta* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we predict an increase in future potential suitable climatic conditions for the Oklahoma panhandle. Maps were generated using MaxEnt.

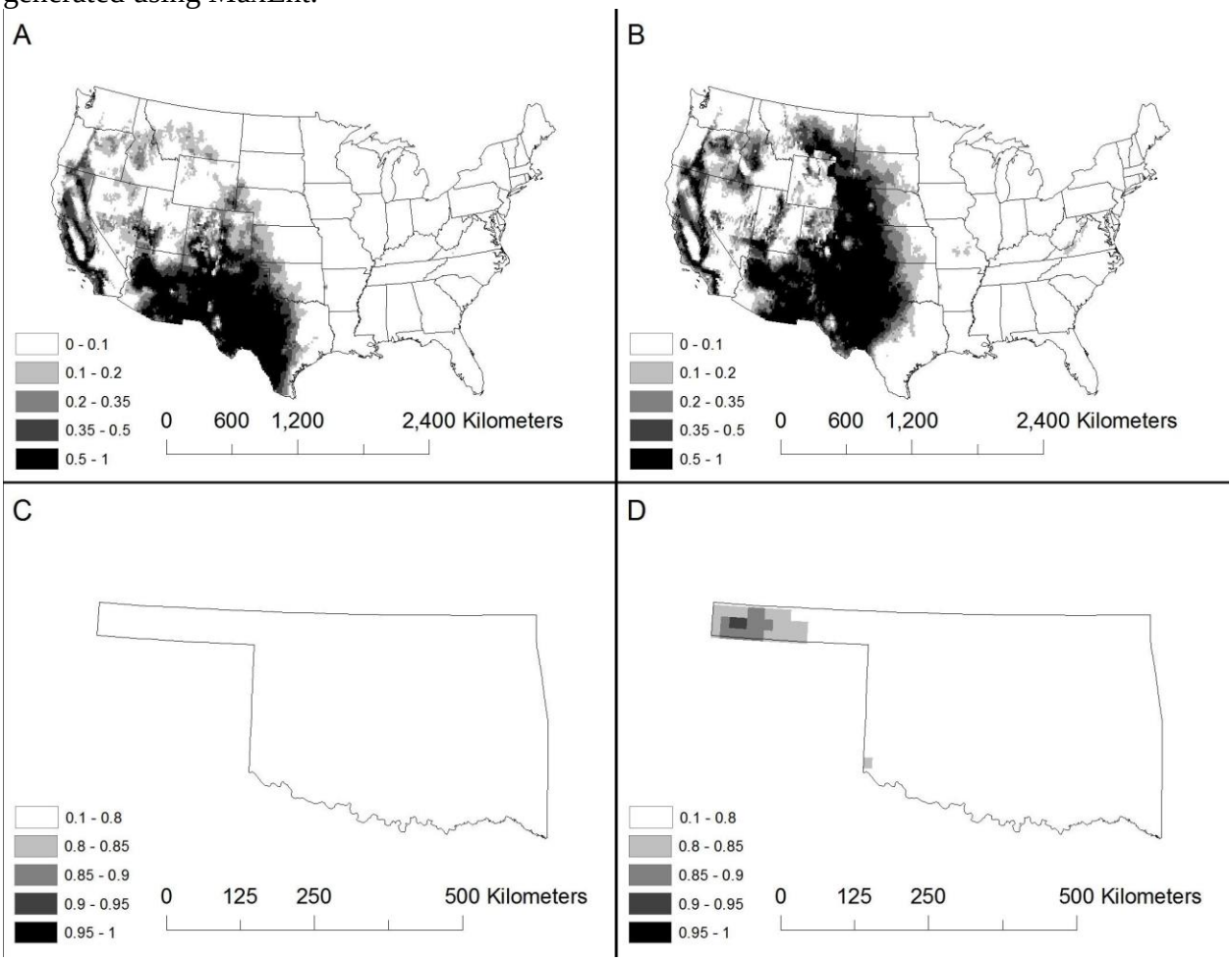

Figure 6. The current potential distribution of *Triatoma sanguisuga* in the United States (A), and the potential distribution by 2070 (B), the current potential distribution in Oklahoma (C), and the potential distribution in OK by 2070 (D). Using a finer resolution for OK, we predict an increase in future potential suitable climatic conditions statewide. Maps were generated using MaxEnt.

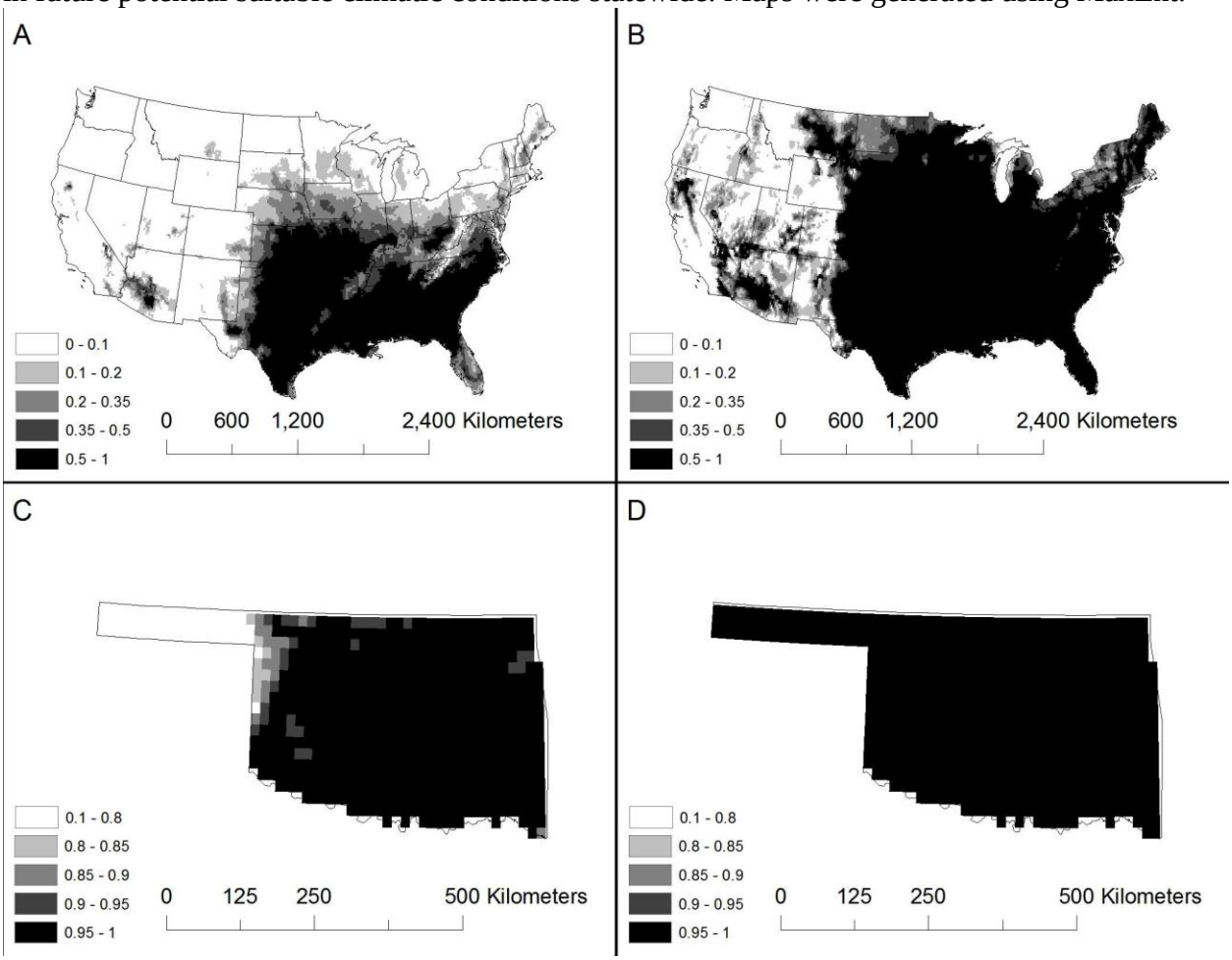

Table 1. Summary of bioclimatic variables used in this study.

Variable	Definition
BIO 1	Annual mean temperature
BIO 2	Mean diurnal range (mean of monthly [max temp – min temp])
BIO 3	Isothermality (BIO 2 / BIO 7) x 100
BIO 4	Temperature seasonality (standard deviation x 100)
BIO 5	Max temperature of warmest month
BIO 6	Min temperature of coldest month
BIO 7	Temperature annual range (BIO 5 – BIO 6)
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 11	Mean temperature of coldest quarter
BIO 12`	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 15	Precipitation seasonality (coefficient of variation)
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
BIO 18	Precipitation of warmest quarter
BIO 19	Precipitation of coldest quarter
Elevation	Elevation above sea level

Table 2. Mean ± standard deviation projected suitability for the study species. Data generated using MaxEnt.

	Current – lower 48	Current – Oklahoma	2070 RCP 8.5 – lower 48 states	2070 RCP 8.5 – Oklahoma
Trypanosoma cruzi	0.359 ± 0.389	0.995 ± 0.025	0.818 ± 0.323	1.000 ± 0.000
Triatoma gerstaeckeri	0.052 ± 0.135	0.110 ± 0.068	0.084 ± 0.158	0.332 ± 0.162
Triatoma indictiva	0.077 ± 0.186	0.569 ± 0.240	0.249 ± 0.308	0.844 ± 0.229
Triatoma lecticularia	0.138 ± 0.203	0.453 ± 0.142	0.333 ± 0.303	0.837 ± 0.093
Triatoma protracta	0.128 ± 0.218	0.161 ± 0.159	0.175 ± 0.252	0.299 ± 0.255
Triatoma sanguisuga	0.263 ± 0.324	0.932 ± 0.188	0.597 ± 0.405	1.000 ± 0.002

GENERAL SUMMARY

This research adds to the exciting and expanding body of work on the epidemiology of *Trypanosoma cruzi, Triatoma* insect vectors, reservoir hosts, and disease surveillance methodologies for Oklahoma. The seven species studied, *T. cruzi, Tadarida brasiliensis, Triatoma gerstaeckeri, T. indictiva, T. lecticularia, T. protracta,* and *T. sanguisuga* are ecologically and economically important in the United States. This section highlights summaries of each chapter and recommendations for future studies.

I present the first report of a wild bat naturally infected with *T. cruzi* in Oklahoma, the second report of a bat naturally infected in the United States, and the fourth reported animal infection in the state (Fox et al. 1986; John and Hoppe 1986; Bradley et al. 2000; Hodo et al. 2016; Chapter 2). I present the first report of *T. cruzi* detection from bat patagia and detail a convenient and sensitive methodology for *T. cruzi* disease surveillance that can be applied to a variety of potential reservoir hosts in underrepresented and endemic areas. I suggest that the migratory Mexican free-tailed bats (MFT; *Tadarida brasiliensis*) potentially contributes to the endemicity of *T. cruzi* in Oklahoma and might contribute to future enzootic expansion. I suggest MFT might play a unique role in the epidemiology of *T. cruzi* through their annual migration from historical endemic foci to novel endemic foci. This claim also is supported by the high prevalence of *T. cruzi* infected triatomines and sylvatic transmission cycles in Texas mammals along the MFT migratory pathway (Kjos et al. 2009; Curtis-Robles et al. 2015, 2016). Furthermore, my findings support the bat seeding hypothesis, which suggests bats are the original reservoir hosts for *T. cruzi* and can establish new endemic transmission cycles through their migration and wide distribution (Stadelmann et al. 2007; Flores-Lopez and Machado 2011; Hamilton et al. 2012a, 2012b; Dario et al. 2017).

Current trends suggest global climate change will facilitate an expansion of tropical diseases, notably vector-borne diseases, throughout temperate regions (Epstein 2000; Lafferty 2009). Because *T. cruzi* is a neglected tropical parasite that persists in temperate regions, such as Oklahoma, it is crucial for disease surveillance efforts to detail current and future regions that present favorable climatic conditions for *T. cruzi* and vector establishment. I used the program MaxEnt to develop an ecological niche model for *T. cruzi* and five widespread *Triatoma* vectors based on 19 bioclimatic variables and 546 published localities within the United States (Chapter 3). My ecological niche model indicates an expansion in potential suitable climatic conditions within the United States for *T. cruzi* and the five *Triatoma* species included in this study. I observed an increase in future potential favorable climatic conditions in Oklahoma for all study species except *T. gerstaeckeri* (Chapter 3). The bioclimatic variables that contributed the most to predicting the potential distribution of *T. cruzi* and the *Triatoma* vectors were annual mean temperature, mean diurnal range, annual precipitation, max temperature of the warmest month, and precipitation of the warmest quarter. Elevating temperatures can directly increase vector-borne pathogen development, transmission season, and morbidity (Marinkelle and Rodriguez 1968; Kutz et al. 2005; Poulin 2006; Lal et al. 2012; Elliot et al. 2015). Increases in suitable habitat, disease morbidity, and inadequate disease surveillance promotes the expansion of *T. cruzi* and important *Triatoma* vectors through Oklahoma and the United States. Without improved disease surveillance, *T. cruzi* may increase in prevalence within wildlife, domestic, and human populations in underrepresented areas in the United States.

Future studies should compare infection rates of important vectors, consider the post-feeding defecation latency times of each vector, consider human dwellings and peridomestic animals for precise areas of high-risk transmission potential, which are likely areas of poor

housing where vectors can readily colonize, focus on assessing *T. cruzi* prevalence in wild and domestic mammalian reservoirs along the southern United States, and improve surveillance of classic and potentially novel arthropod vectors. Lastly, I urge physicians, veterinarians, public health officials, and researchers to increase disease surveillance for *T. cruzi* and triatomine vectors to better understand the current and future epidemiology of *T. cruzi*, triatomine vectors, and reservoir hosts in the United States.

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APPENDICES

Appendix A1. Supplementary table for field sampling distribution of the 361 Mexican free-tailed bats (*Tadarida brasiliensis*) during the summer of 2017 (Chapter 2).

Month	*County*	*Amount Sampled*	*Number of Females*	*Number of Males*	*Age*	*Female Reproductive Status*	*Number Positive for T. cruzi*
May	Woodward	20	20	0	Adult	Pregnant	0
May	Woods	39	39	0	Adult	Pregnant	0
May	Major	32	32	0	Adult	Pregnant	0
June	Woodward	46	46	0	Adult	Lactating	0
June	Woods	64	62	2	Adult	Lactating	0
June	*Major*	32	32	0	Adult	Lactating	0
July	Woodward	32	31	1	Adult	Lactating	0
July	Woods	32	27	5	Adult	Lactating	0
August	Woodward	32	19	13	Juvenile	Immature	1
August	Woods	32	10	22	Juvenile	Immature	0
Total	*3*	*361*	*318*	*43*	*Adult + Juvenile*	*Pregnant + Lactating + Immature*	*1*

Appendix A2. Location data of *Trypanosoma cruzi* found in intermediate or definitive hosts (Chapter 3). Total number of published records is 215.

Species	Latitude	Longitude	Location	Source
T. cruzi	29.4201	-98.5721	Bexar County, TX	Kjos et al. 2008
T. cruzi	30.6504	-96.3226	Brazos County, TX	Kjos et al. 2008
T. cruzi	30.2097	-97.6982	Travis County, TX	Kjos et al. 2008
T. cruzi	29.3343	-99.8125	Uvalde County, TX	Kjos et al. 2008
T. cruzi	27.7842	-98.0465	Jim Wells County, TX	Kjos et al. 2008
T. cruzi	27.7693	-97.4814	Nueces County, TX	Kjos et al. 2008
T. cruzi	30.7508	-104.1931	Jeff Davis County, Tx	Kjos et al. 2008
T. cruzi	28.4117	-99.8125	Dimmit County, TX	Kjos et al. 2008
T. cruzi	27.8174	-99.0129	Webb County, TX	Kjos et al. 2008
T. cruzi	27.0273	-98.2213	Brooks County, TX	Kjos et al. 2008
T. cruzi	27.4308	-97.6982	Kleberg County, TX	Kjos et al. 2008
T. cruzi	28	-97.5247	San Patricio County, TX	Kjos et al. 2008
T. cruzi	28.3624	-97.6982	Bee County, TX	Kjos et al. 2008
T. cruzi	28.9089	-97.8722	Karnes County, TX	Kjos et al. 2008
T. cruzi	29.1235	-97.3517	Dewitt County, TX	Kjos et al. 2008
T. cruzi	28.8661	-98.5721	Atascosa County, TX	Kjos et al. 2008
T. cruzi	28.8314	-99.1013	Frio County, TX	Kjos et al. 2008
T. cruzi	29.2988	-99.0129	Medina County, TX	Kjos et al. 2008
T. cruzi	29.7404	-99.2786	Bandera County, TX	Kjos et al. 2008
T. cruzi	30.0945	-99.4562	Kerr County, TX	Kjos et al. 2008
T. cruzi	29.9603	-98.7481	Kendall County, TX	Kjos et al. 2008
T. cruzi	29.8106	-98.2213	Comal County, TX	Kjos et al. 2008
T. cruzi	30.3501	-98.3965	Blanco County, TX	Kjos et al. 2008
T. cruzi	30.0538	-98.0029	Hays County, TX	Kjos et al. 2008
T. cruzi	30.7592	-97.6982	Williamson County, TX	Kjos et al. 2008
T. cruzi	30.727	-98.2213	Burnet County, TX	Kjos et al. 2008
T. cruzi	31.2738	-98.2213	Lampasas County, TX	Kjos et al. 2008
T. cruzi	31.4774	-97.8722	Coryell County, TX	Kjos et al. 2008
T. cruzi	31.5182	-97.179	Mclennan County, TX	Kjos et al. 2008
T. cruzi	31.7705	-98.9245	Brown County, TX	Kjos et al. 2008
T. cruzi	31.8715	-99.9912	Runnels County, TX	Kjos et al. 2008
T. cruzi	32.2548	-97.7417	Somervell County, TX	Kjos et al. 2008
T. cruzi	32.7732	-97.3517	Tarrant County, TX	Kjos et al. 2008
T. cruzi	32.7767	-96.797	Dallas County, TX	Kjos et al. 2008
T. cruzi	32.1321	-95.8143	Henderson County, TX	Kjos et al. 2008
T. cruzi	30.483	-95.9833	Grimes County, TX	Kjos et al. 2008

T. cruzi	30.6815	-95.6458	Walker County, TX	Kjos et al. 2008
T. cruzi	30.3213	-95.4778	Montgomery County, TX	Kjos et al. 2008
T. cruzi	29.7752	-95.3103	Harris County, TX	Kjos et al. 2008
T. cruzi	28.8205	-95.9833	Matagorda County, TX	Kjos et al. 2008
T. cruzi	29.8165	-94.1514	Jefferson County, TX	Kjos et al. 2008
T. cruzi	28.7713	-97.0068	Victoria County, TX	Kjos et al. 2008
T. cruzi	33.6754	-101.798	Lubbock County, TX	Kjos et al. 2008
T. cruzi	27.8006	-97.3964	Corpus Christi, TX	Roellig et al. 2008
T. cruzi	29.9511	-90.0715	New Orleans, LA	Roellig et al. 2008
T. cruzi	37.7354	-120.3839	Lake Don Pedro, CA	Roellig et al. 2008
T. cruzi	26.1837	-98.1231	Alamo, TX	Roellig et al. 2008
T. cruzi	35.4123	-85.9717	Hillsboro, TN	Roellig et al. 2008
T. cruzi	36.7473	-95.9808	Bartlesville, OK	Roellig et al. 2008
T. cruzi	32.4488	-81.7832	Statesboro, GA	Roellig et al. 2008
T. cruzi	31.8691	-81.609	Fort Stewart, GA	Roellig et al. 2008
T. cruzi	30.5185	-84.2519	Maclay State Park, FL	Roellig et al. 2008
T. cruzi	27.9506	-82.4572	Tampa, FL	Roellig et al. 2008
T. cruzi	31.9994	-81.1196	Chatham County, GA	Roellig et al. 2008
T. cruzi	33.9069	-83.3572	White Hall, GA	Roellig et al. 2008
T. cruzi	32.6099	-85.4808	Auburn, AL	Roellig et al. 2008
T. cruzi	32.4242	-82.0843	Candler County, GA	Roellig et al. 2008
T. cruzi	30.4383	-84.2807	Tallahassee, FL	Roellig et al. 2008
T. cruzi	30.6563	-84.2089	Tall Timbers, FL	Roellig et al. 2008
T. cruzi	30.5665	-84.9478	Torreya State Park, FL	Roellig et al. 2008
T. cruzi	30.4353	-84.5668	Lake Talquin, FL	Roellig et al. 2008
T. cruzi	34.9046	-82.6483	Pickens County, SC	Roellig et al. 2008
T. cruzi	31.7615	-81.1086	Ossabaw Island, GA	Roellig et al. 2008
T. cruzi	33.8848	-83.3577	Whitehall Forest, GA	Roellig et al. 2008
T. cruzi	33.9519	-83.3576	Athens, GA	Roellig et al. 2008
T. cruzi	30.9638	-81.7226	Woodbine, GA	Roellig et al. 2008
T. cruzi	31.708	-81.7423	Ludowici, GA	Roellig et al. 2008
T. cruzi	34.2976	-83.1614	Victoria Bryant State Park, GA	Roellig et al. 2008
T. cruzi	31.9349	-81.0471	Skidaway Island, GA	Roellig et al. 2008
T. cruzi	39.0993	-76.8483	Laurel, MD	Roellig et al. 2008
T. cruzi	31.6289	-81.1527	St. Catherine's Island, GA	Roellig et al. 2008
T. cruzi	35.7724	-86.3377	Rutherford County, TN	Roellig et al. 2008
T. cruzi	29.6516	-82.3248	Gainesville, FL	Roellig et al. 2008
T. cruzi	32.3596	-81.7787	Bulloch County, GA	Roellig et al. 2008
T. cruzi	33.749	-84.388	Atlanta, GA	Roellig et al. 2008
T. cruzi	31.7619	-106.485	El Paso County, TX	Kjos et al. 2009

T. cruzi	29.663	-103.3587	Brewster County, TX	Kjos et al. 2009
T. cruzi	30.2349	-102.1633	Terrell County, TX	Kjos et al. 2009
T. cruzi	29.8688	-101.1617	Val Verde County, TX	Kjos et al. 2009
T. cruzi	31.3839	-100.4397	Tom Green County, TX	Kjos et al. 2009
T. cruzi	30.9802	-99.8125	Menard County, TX	Kjos et al. 2009
T. cruzi	29.2935	-100.3498	Kinney County, TX	Kjos et al. 2009
T. cruzi	28.741	-100.3498	Maverick County, TX	Kjos et al. 2009
T. cruzi	28.8801	-99.7233	Zavala County, TX	Kjos et al. 2009
T. cruzi	28.2759	-99.1013	La Salle County, TX	Kjos et al. 2009
T. cruzi	27.7525	-98.5721	Duval County, TX	Kjos et al. 2009
T. cruzi	26.6215	-98.7481	Starr County, TX	Kjos et al. 2009
T. cruzi	26.4656	-98.2213	Hidalgo County, TX	Kjos et al. 2009
T. cruzi	26.4948	-97.6982	Willacy County, TX	Kjos et al. 2009
T. cruzi	26.1285	-97.5247	Cameron County, TX	Kjos et al. 2009
T. cruzi	28.3465	-97.1359	Refugio County, TX	Kjos et al. 2009
T. cruzi	28.4169	-96.6638	Calhoun County, TX	Kjos et al. 2009
T. cruzi	29.3359	-96.8351	Lavaca County, TX	Kjos et al. 2009
T. cruzi	31.0688	-95.1432	Trinity County, TX	Kjos et al. 2009
T. cruzi	29.8851	-99.8125	Real County, TX	Kjos et al. 2009
T. cruzi	30.315	-98.9245	Gillespie County, TX	Kjos et al. 2009
T. cruzi	31.1344	-97.5247	Bell County, TX	Kjos et al. 2009
T. cruzi	30.0459	-97.3517	Bastrop County, TX	Kjos et al. 2009
T. cruzi	30.8093	-96.9795	Milam County, TX	Kjos et al. 2009
T. cruzi	32.0992	-96.493	Navarro County, TX	Kjos et al. 2009
T. cruzi	32.7157	-117.1611	Murray Canyon, San Diego County, CA	Wood 1941
T. cruzi	34.0522	-118.2437	Eaton Canyon, Los Angeles County	Wood 1941
T. cruzi	32.7098	-108.302	Tyrone, NM	Wood 1941
T. cruzi	32.9691	-108.2378	Grant County, NM	Wood 1941
T. cruzi	29.27	-103.3	Chisos Mountains, TX	Wood 1941
T. cruzi	30.2052	-103.2446	Marathon, TX	Wood 1941
T. cruzi	28.948	-100.624	Quemado, TX	Wood 1941
T. cruzi	29.3475	-99.1414	Hondo, TX	Wood 1941
T. cruzi	36.7344	-95.6458	Nowata County, OK	Bradley et al. 2000
T. cruzi	34.8622	-94.645	Le Flore County, OK	Bradley et al. 2000
T. cruzi	34.9879	-95.8143	Pittsburg County, OK	Bradley et al. 2000
T. cruzi	32.3547	-89.3985	Mississippi	Cantey et al. 2012
T. cruzi	31.0982	-97.3428	Temple, Texas	Packchanian 1940
T. cruzi	32.4088	-83.3789	Bleckley County, GA	Pung et al. 1995
T. cruzi	33.9021	-96.3226	Bryan County, GA	Pung et al. 1995
T. cruzi	30.1634	-94.8106	Liberty County, GA	Pung et al. 1995

T. cruzi	32.7075	-81.6035	Screven County, GA	Pung et al. 1995
T. cruzi	34.5528	-82.6483	Anderson County, SC	Yabsley and Noblet 2002
T. cruzi	34.6613	-81.6035	Union County, SC	Yabsley and Noblet 2002
T. cruzi	34.3091	-81.6035	Newberry County, SC	Yabsley and Noblet 2002
T. cruzi	34.0795	-82.8641	Elbert County, GA	Yabsley and Noblet 2002
T. cruzi	33.8083	-82.7779	Wilkes County, GA	Yabsley and Noblet 2002
T. cruzi	33.9519	-83.3576	Clarke County, GA	Yabsley and Noblet 2002
T. cruzi	33.912	-83.0361	Oglethorpe County, GA	Yabsley and Noblet 2002
T. cruzi	33.7875	-82.4319	Lincoln County, GA	Yabsley and Noblet 2002
T. cruzi	34.0412	-80.9429	Richland County, SC	Yabsley and Noblet 2002
T. cruzi	33.8839	-89.3227	Calhoun County, SC	Yabsley and Noblet 2002
T. cruzi	33.6006	-81.6035	Aiken County, SC	Yabsley and Noblet 2002
T. cruzi	32.4914	-81.0755	Jasper County, SC	Yabsley and Noblet 2002
T. cruzi	32.8085	-81.1196	Hampton County, SC	Yabsley and Noblet 2002
T. cruzi	32.7957	-79.7848	Charleston County, SC	Yabsley and Noblet 2002
T. cruzi	33.9197	-78.9288	Horry County, SC	Yabsley and Noblet 2002
T. cruzi	35.1619	-85.1479	Hamilton County, TN	Maloney et al. 2010
T. cruzi	35.0929	-85.6435	Marion County, TN	Maloney et al. 2010
T. cruzi	35.5194	-84.7942	Meigs County, TN	Maloney et al. 2010
T. cruzi	36.4446	-82.9502	Hawkins County, TN	Maloney et al. 2010
T. cruzi	36.3321	-82.5186	Washington County, TN	Maloney et al. 2010
T. cruzi	36.1348	-82.821	Greene County, TN	Maloney et al. 2010
T. cruzi	36.4933	-82.3452	Sullivan County, TN	Maloney et al. 2010
T. cruzi	36.2054	-83.2934	Hamblen County, TN	Maloney et al. 2010
T. cruzi	35.8361	-84.5641	Roane County, TN	Maloney et al. 2010
T. cruzi	32.7157	-117.1611	San Diego, CA	Kofoid and McCulloch 1916
T. cruzi	32.5889	-85.3963	Lee County, AL	Olsen et al. 1964
T. cruzi	31.8173	-85.355	Barbour County, AL	Olsen et al. 1964
T. cruzi	32.3731	-85.6846	Macon County, AL	Olsen et al. 1964
T. cruzi	33.2682	-85.52	Randolph County, AL	Olsen et al. 1964
T. cruzi	31.1636	-97.0068	Falls County, TX	Curtis-Robles et al. 2016
T. cruzi	29.8896	-96.8351	Fayette County, TX	Curtis-Robles et al. 2016
T. cruzi	29.5379	-96.493	Colorado County, TX	Curtis-Robles et al. 2016
T. cruzi	31.6137	-98.5721	Mills County, TX	Curtis-Robles et al. 2016
T. cruzi	31.6369	-98.2213	Hamilton County, TX	Curtis-Robles et al. 2016
T. cruzi	32.1793	-98.2213	Erath County, TX	Curtis-Robles et al. 2016
T. cruzi	31.8508	-97.6982	Bosque County, TX	Curtis-Robles et al. 2016
T. cruzi	32.7512	-98.3104	Palo Pinto County, TX	Curtis-Robles et al. 2016
T. cruzi	31.008	-96.493	Robertson County, TX	Curtis-Robles et al. 2016
T. cruzi	30.2353	-96.3652	Washington County, TX	Curtis-Robles et al. 2016
T. cruzi	30.2967	-96.9639	Lee County, TX	Curtis-Robles et al. 2016

T. cruzi	31.2816	-84.4803	Baker County, GA	Brown et al. 2010
T. cruzi	34.3646	-83.2078	Franklin County, GA	Brown et al. 2010
T. cruzi	31.2624	-81.6035	Glynn County, GA	Brown et al. 2010
T. cruzi	30.8417	-83.8473	Thomas County, GA	Brown et al. 2010
T. cruzi	32.013	-84.5641	Webster County, GA	Brown et al. 2010
T. cruzi	26.6105	-81.0755	Hendry County, FL	Brown et al. 2010
T. cruzi	30.4906	-84.1857	Leon County, FL	Brown et al. 2010
T. cruzi	30.1302	-84.3542	Wakulla County, FL	Brown et al. 2010
T. cruzi	34.0489	-111.0937	Arizona	Brown et al. 2010
T. cruzi	36.7783	-119.4179	California	Brown et al. 2010
T. cruzi	27.6648	-81.5158	Florida	Brown et al. 2010
T. cruzi	32.1656	-82.9001	Georgia	Brown et al. 2010
T. cruzi	37.9643	-91.8318	Missouri	Brown et al. 2010
T. cruzi	37.4316	-78.6569	Virginia	Brown et al. 2010
T. cruzi	36.9886	-86.4997	Warren County, KY	Groce 2008
T. cruzi	36.9677	-85.8486	Barren County, KY	Groce 2008
T. cruzi	38.7849	-76.8721	Prince George's County, MD	Herman and Bruce 1962
T. cruzi	36.12	-80.1875	Forsyth County, NC	Karsten et al. 1992
T. cruzi	36.1593	-95.941	Tulsa, OK	John and Hoppe 1986
T. cruzi	33.0338	-83.2934	Baldwin County, GA	Parrish and Mead 2010
T. cruzi	30.4515	-91.1871	Baton Rouge, LA	Barr et al. 1991
T. cruzi	28.326	-99.4076	Chaparral WMA, TX	Pinto et al. 2010
T. cruzi	32.0575	-111.6661	Pima County, AZ	Wood 1952
T. cruzi	37.0454	-121.958	Santa Cruz County, AZ	Wood 1952
T. cruzi	34.4442	-117.9353	Juniper Hills, CA	Wood 1975
T. cruzi	37.2519	-119.6963	Madera County, CA	Wood 1962
T. cruzi	21.8853	-102.2916	Aguascalientes	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	30.8406	-115.2838	Baja California	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	26.0444	-111.6661	Baja California Sur	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.83803	-90.5277	Campeche	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	16.7569	-93.1292	Chiapas	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	28.4854	-105.7821	Chihuahua	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	27.0587	-101.7068	Coahuila	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.1223	-104.0072	Colima	Cruz-Reyes and Pickering-Lopez 2006

T. cruzi	19.4326	-99.1332	Distrito Federal	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	37.2753	-107.8801	Durango	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.4969	-99.7233	Estado de Mexico	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	20.917	-101.1617	Guanajuato	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	17.4392	-99.5451	Guerrero	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	20.0911	-98.7624	Hidalgo	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	20.6595	-103.3494	Jalisco	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.5665	-101.7068	Michoacan	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	18.6813	-99.1013	Morelos	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	21.7514	-104.8455	Nayarit	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	25.5922	-99.9962	Nuevo Leon	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	17.0542	-96.7132	Oaxaca	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.0414	-98.2063	Puebla	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	20.5888	-100.3899	Queretaro	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.1817	-88.4791	Quintana Roo	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	22.1566	-100.9855	San Luis Potosi	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	25.1721	-107.4795	Sinaloa	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	29.2972	-110.3309	Sonora	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	17.8409	-92.6189	Tabasco	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	24.2669	-98.8363	Tamaulipas	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.3182	-98.2375	Tlaxcala	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	19.2602	-96.5783	Veracruz	Cruz-Reyes and Pickering-Lopez 2006
T. cruzi	18.8067	-89.3985	Yucatan	Cruz-Reyes and Pickering-Lopez 2006

T. cruzi	22.7709	-102.5832	Zacatecas	Cruz-Reyes and Pickering-Lopez 2006

Appendix A3. Location data for the five *Triatoma* species included in this study (Chapter 3).

Total number of published records is 331.

T. gerstaeckeri	26.1326	-97.6311	San Benito, TX	Beard et al. 2003
T. gerstaeckeri	26.1285	-97.5247	Cameron County, TX	Burkholder et al. 1980
T. gerstaeckeri	26.4656	-98.2213	Hidalgo County, TX	Burkholder et al. 1980
T. gerstaeckeri	31.3478	-104.4723	Culberson County, TX	Kjos et al. 2009
T. gerstaeckeri	31.4308	-103.7289	Reeves County, TX	Kjos et al. 2009
T. gerstaeckeri	30.7508	-104.1931	Jeff Davis County, TX	Kjos et al. 2009
T. gerstaeckeri	29.9481	-104.1001	Presidio County, TX	Kjos et al. 2009
T. gerstaeckeri	29.663	-103.3587	Brewster County, TX	Kjos et al. 2009
T. gerstaeckeri	30.2349	-102.1633	Terrell County, TX	Kjos et al. 2009
T. gerstaeckeri	29.8688	-101.1617	Val Verde County, TX	Kjos et al. 2009
T. gerstaeckeri	31.3839	-100.4397	Tom Green County, TX	Kjos et al. 2009
T. gerstaeckeri	30.9802	-99.8125	Menard County, TX	Kjos et al. 2009
T. gerstaeckeri	29.2935	-100.3498	Kinney County, TX	Kjos et al. 2009
T. gerstaeckeri	28.741	-100.3498	Maverick County, TX	Kjos et al. 2009
T. gerstaeckeri	29.8851	-99.8125	Real County, TX	Kjos et al. 2009
T. gerstaeckeri	30.0945	-99.4562	Kerr County, TX	Kjos et al. 2009
T. gerstaeckeri	29.7404	-99.2786	Bandera County, TX	Kjos et al. 2009
T. gerstaeckeri	29.3343	-99.8125	Uvalde County, TX	Kjos et al. 2009
T. gerstaeckeri	28.8801	-99.7233	Zavala County, TX	Kjos et al. 2009
T. gerstaeckeri	28.8314	-99.1013	Frio County, TX	Kjos et al. 2009
T. gerstaeckeri	29.2988	-99.0129	Medina County, TX	Kjos et al. 2009

T. gerstaeckeri	34.5509	-102.3119	Dimmitt County, TX	Kjos et al. 2009
T. gerstaeckeri	27.8174	-99.0129	Webb County, TX	Kjos et al. 2009
T. gerstaeckeri	26.6215	-98.7481	Starr County, TX	Kjos et al. 2009
T. gerstaeckeri	26.4948	-97.6982	Willacy County, TX	Kjos et al. 2009
T. gerstaeckeri	27.7525	-98.5721	Duval County, TX	Kjos et al. 2009
T. gerstaeckeri	27.7842	-98.0465	Jim Wells County, TX	Kjos et al. 2009
T. gerstaeckeri	27.4308	-97.6982	Kleberg County, TX	Kjos et al. 2009
T. gerstaeckeri	27.7693	-97.4814	Nueces County, TX	Kjos et al. 2009
T. gerstaeckeri	28	-97.5247	San Patricio County, TX	Kjos et al. 2009
T. gerstaeckeri	28.2759	-99.1013	La Salle County, TX	Kjos et al. 2009
T. gerstaeckeri	28.3102	-98.5721	Mcmullen County, TX	Kjos et al. 2009
T. gerstaeckeri	31.2738	-98.2213	Lampasas County, TX	Kjos et al. 2009
T. gerstaeckeri	30.727	-98.2213	Burnet County, TX	Kjos et al. 2009
T. gerstaeckeri	31.1344	-97.5247	Bell County, TX	Kjos et al. 2009
T. gerstaeckeri	31.5182	-97.179	Mclennan County, TX	Kjos et al. 2009
T. gerstaeckeri	32.0622	-97.179	Hill County, TX	Kjos et al. 2009
T. gerstaeckeri	32.1321	-95.8143	Henderson County, TX	Kjos et al. 2009
T. gerstaeckeri	32.5539	-94.3154	Harrison County, TX	Kjos et al. 2009
T. gerstaeckeri	30.6504	-96.3226	Brazos County, TX	Kjos et al. 2009
T. gerstaeckeri	30.8093	-96.9795	Milam County, TX	Kjos et al. 2009
T. gerstaeckeri	29.3359	-96.8351	Lavaca County, TX	Kjos et al. 2009
T. gerstaeckeri	34.4985	-102.3464	Castro County, TX	Sarkar et al. 2010
T. gerstaeckeri	29.3763	-94.8521	Galveston County, TX	Sarkar et al. 2010

T. gerstaeckeri	29.4835	-97.5247	Gonzales County, TX	Sarkar et al. 2010
T. gerstaeckeri	33.6754	-101.798	Lubbock County, TX	Sarkar et al. 2010
T. gerstaeckeri	32.7416	-97.8722	Parker County, TX	Sarkar et al. 2010
T. gerstaeckeri	28.7713	-97.0068	Victoria County, TX	Sarkar et al. 2010
T. gerstaeckeri	29.2684	-98.0465	Wilson County, TX	Sarkar et al. 2010
T. gerstaeckeri	26.9731	-99.1013	Zapata County, TX	Sarkar et al. 2010
T. gerstaeckeri	29.4201	-98.5721	Bexar County, TX	Sullivan et al. 1949
T. gerstaeckeri	29.8849	-97.6699	Caldwell County, TX	Sullivan et al. 1949
T. gerstaeckeri	28.4169	-96.6638	Calhoun County, TX	Sullivan et al. 1949
T. gerstaeckeri	29.1235	-97.3517	Dewitt County, TX	Sullivan et al. 1949
T. gerstaeckeri	29.6477	-97.8722	Guadalupe County, TX	Sullivan et al. 1949
T. gerstaeckeri	30.0538	-98.0029	Hays County, TX	Sullivan et al. 1949
T. gerstaeckeri	28.3465	-97.1359	Refugio County, TX	Sullivan et al. 1949
T. gerstaeckeri	30.2097	-97.6982	Travis County, TX	Sullivan et al. 1949
T. gerstaeckeri	30.7592	-97.6982	Williamson County, TX	Sullivan et al. 1949
T. gerstaeckeri	28.948	-100.624	Quemado, TX	Wood 1941
T. gerstaeckeri	29.3475	-99.1414	Hondo, TX	Wood 1941
T. gerstaeckeri	26.5257	-99.1096	Salineno, TX	Wood 1941
T. gerstaeckeri	26.4103	-98.1353	Faysville, TX	Wood 1941
T. gerstaeckeri	27.5989	-98.4081	Benavides, TX	Wood 1941
T. gerstaeckeri	27.4448	-98.5283	Realitos, TX	Wood 1941
T. gerstaeckeri	27.4486	-99.0873	Aguilares, TX	Wood 1941
T. gerstaeckeri	27.8174	-99.0129	Webb, TX	Wood 1941

T. gerstaeckeri	28.3455	-99.6134	Catarina, TX	Wood 1941
T. gerstaeckeri	28.4436	-99.7589	Asherton, TX	Wood 1941
T. gerstaeckeri	30.1424	-102.394	Sanderson, TX	Wood 1941
T. indictiva	30.2349	-102.1633	Terrell County, TX	Kjos et al. 2009
T. indictiva	33.5779	-101.8552	Lubbok County, TX	Kjos et al. 2009
T. indictiva	31.3839	-100.4397	Tom Green County, TX	Kjos et al. 2009
T. indictiva	30.0945	-99.4562	Kerr County, TX	Kjos et al. 2009
T. indictiva	30.315	-98.9245	Gillespie County, TX	Kjos et al. 2009
T. indictiva	30.6925	-98.7481	Llano County, TX	Kjos et al. 2009
T. indictiva	30.3501	-98.3965	Blanco County, TX	Kjos et al. 2009
T. indictiva	29.9603	-98.7481	Kendall County, TX	Kjos et al. 2009
T. indictiva	29.8106	-98.2213	Comal County, TX	Kjos et al. 2009
T. indictiva	30.2097	-97.6982	Travis County, TX	Kjos et al. 2009
T. indictiva	27.5989	-98.4081	Benavides, TX	Wood 1941
T. indictiva	32.7098	-108.302	Tyrone, NM	Wood 1941
T. lecticularia	35.6038	-97.3517	Oklahoma County, OK	Usinger 1944; Drew and Schaefer 1962
T. lecticularia	33.931	-98.7481	Wichita County, TX	Kjos et al. 2009
T. lecticularia	32.7732	-97.3517	Tarrant County, TX	Kjos et al. 2009
T. lecticularia	32.1793	-98.2213	Erath County, TX	Kjos et al. 2009
T. lecticularia	31.2389	-98.7481	San Saba County, TX	Kjos et al. 2009
T. lecticularia	32.0992	-96.493	Navarro County, TX	Kjos et al. 2009
T. lecticularia	31.2153	-95.9833	Leon County, TX	Kjos et al. 2009

T. lecticularia	30.8093	-96.9795	Milam County, TX	Kjos et al. 2009
T. lecticularia	31.0688	-95.1432	Trinity County, TX	Kjos et al. 2009
T. lecticularia	30.7151	-94.8106	Polk County, TX	Kjos et al. 2009
T. lecticularia	30.3213	-95.4778	Montgomery County, TX	Kjos et al. 2009
T. lecticularia	30.6504	-96.3226	Brazos County, TX	Kjos et al. 2009
T. lecticularia	31.5182	-97.179	Mclennan County, TX	Kjos et al. 2009
T. lecticularia	31.1344	-97.5247	Bell County, TX	Kjos et al. 2009
T. lecticularia	30.7592	-97.6982	Williamson County, TX	Kjos et al. 2009
T. lecticularia	30.2097	-97.6982	Travis County, TX	Kjos et al. 2009
T. lecticularia	29.1235	-97.3517	Dewitt County, TX	Kjos et al. 2009
T. lecticularia	29.2988	-99.0129	Medina County, TX	Kjos et al. 2009
T. lecticularia	29.3343	-99.8125	Uvalde County, TX	Kjos et al. 2009
T. lecticularia	28.8801	-99.7233	Zavala County, TX	Kjos et al. 2009
T. lecticularia	29.1235	-97.3517	Dimmitt County, TX	Kjos et al. 2009
T. lecticularia	27.8174	-99.0129	Webb County, TX	Kjos et al. 2009
T. lecticularia	27.7525	-98.5721	Duval County, TX	Kjos et al. 2009
T. lecticularia	26.4656	-98.2213	Hidalgo County, TX	Kjos et al. 2009
T. lecticularia	26.1285	-97.5247	Cameron County, TX	Kjos et al. 2009
T. lecticularia	34.0489	-111.0937	Arizona	Lent and Wygodzinsky 1979
T. lecticularia	36.7783	-119.4179	California	Lent and Wygodzinsky 1979
T. lecticularia	27.6648	-81.5158	Florida	Lent and Wygodzinsky 1979
T. lecticularia	32.1656	-82.9001	Georgia	Lent and Wygodzinsky 1979
T. lecticularia	40.6331	-89.3985	Illinois	Lent and Wygodzinsky 1979

T. lecticularia	39.0119	-98.4842	Kansas	Lent and Wygodzinsky 1979
T. lecticularia	30.9843	-91.9623	Louisiana	Lent and Wygodzinsky 1979
T. lecticularia	39.0458	-76.6413	Maryland	Lent and Wygodzinsky 1979
T. lecticularia	37.9643	-91.8318	Missouri	Lent and Wygodzinsky 1979
T. lecticularia	34.5199	-105.8701	New Mexico	Lent and Wygodzinsky 1979
T. lecticularia	35.7596	-79.0193	North Carolina	Lent and Wygodzinsky 1979
T. lecticularia	35.0078	-97.0929	Oklahoma	Lent and Wygodzinsky 1979
T. lecticularia	41.2033	-77.1945	Pennsylvania	Lent and Wygodzinsky 1979
T. lecticularia	33.8361	-81.1637	South Carolina	Lent and Wygodzinsky 1979
T. lecticularia	35.5175	-86.5804	Tennessee	Lent and Wygodzinsky 1979
T. lecticularia	31.9686	-99.9018	Texas	Lent and Wygodzinsky 1979
T. lecticularia	30.0459	-97.3517	Bastrop County, TX	Sarkar et al. 2010
T. lecticularia	30.3501	-98.3965	Blanco County, TX	Sarkar et al. 2010
T. lecticularia	30.45	-96.6638	Burleson County, TX	Sarkar et al. 2010
T. lecticularia	33.6754	-101.798	Lubbock County, TX	Sarkar et al. 2010
T. lecticularia	32.7416	-97.8722	Parker County, TX	Sarkar et al. 2010
T. lecticularia	30.7151	-94.8106	Polk County, TX	Sullivan et al. 1949
T. lecticularia	32.2548	-97.7417	Somervell County, TX	Sullivan et al. 1949
T. lecticularia	28.8849	-82.5186	Citrus County, FL	Thurman 1948
T. lecticularia	30.2485	-82.9932	Suwannee County, FL	Thurman 1948
T. lecticularia	34.3672	-80.5883	Kershaw County, SC	Yabsley and Noblet 2002
T. protracta	31.5707	-105.5943	Hudspeth County, TX	Kjos et al. 2009
T. protracta	30.7508	-104.1931	Jeff Davis County, TX	Kjos et al. 2009

T. protracta	31.4308	-103.7289	Reeves County, TX	Kjos et al. 2009
T. protracta	29.9481	-104.1001	Presidio County, TX	Kjos et al. 2009
T. protracta	29.663	-103.3587	Brewster County, TX	Kjos et al. 2009
T. protracta	31.9973	-102.0779	Midland County, TX	Kjos et al. 2009
T. protracta	32.6988	-102.7135	Gaines County, TX	Kjos et al. 2009
T. protracta	33.2115	-102.8975	Yoakum County, TX	Kjos et al. 2009
T. protracta	33.1446	-101.798	Lynn County, TX	Kjos et al. 2009
T. protracta	33.1956	-101.2524	Garza County, TX	Kjos et al. 2009
T. protracta	33.5779	-101.8552	Lubbock County, TX	Kjos et al. 2009
T. protracta	34.902	-101.798	Randall County, TX	Kjos et al. 2009
T. protracta	33.1403	-99.8125	Haskell County, TX	Kjos et al. 2009
T. protracta	33.256	-98.2213	Jack County, TX	Kjos et al. 2009
T. protracta	29.8688	-101.1617	Val Verde County, TX	Kjos et al. 2009
T. protracta	30.4338	-99.8125	Kimble County, TX	Kjos et al. 2009
T. protracta	29.3343	-99.8125	Uvalde County, TX	Kjos et al. 2009
T. protracta	29.2988	-99.0129	Medina County, TX	Kjos et al. 2009
T. protracta	28.741	-100.3498	Maverick County, TX	Kjos et al. 2009
T. protracta	34.5509	-102.3119	Dimmitt County, TX	Kjos et al. 2009
T. protracta	27.8174	-99.0129	Webb County, TX	Kjos et al. 2009
T. protracta	26.9731	-99.1013	Zapata County, TX	Kjos et al. 2009
T. protracta	36.053	-107.9559	Chaco Canyon National Monument, NM	Woods 1975
T. protracta	32.9691	-108.2378	Grant County, NM	Woods 1975

T. protracta	34.0489	-111.0937	Arizona	Lent and Wygodzinsky 1979
T. protracta	36.7783	-119.4179	California	Lent and Wygodzinsky 1979
T. protracta	39.5501	-105.7821	Colorado	Lent and Wygodzinsky 1979
T. protracta	38.8026	-116.4194	Nevada	Lent and Wygodzinsky 1979
T. protracta	34.5199	-105.8701	New Mexico	Lent and Wygodzinsky 1979
T. protracta	31.9686	-99.9018	Texas	Lent and Wygodzinsky 1979
T. protracta	39.321	-111.0937	Utah	Lent and Wygodzinsky 1979
T. protracta	32.3426	-102.7135	Andrews County, TX	Sarkar et al. 2010
T. protracta	29.4201	-98.5721	Bexar County, TX	Sarkar et al. 2010
T. protracta	33.091	-102.3464	Terry County, TX	Sarkar et al. 2010
T. protracta	32.7157	-117.1611	Murray Canyon, San Diego County, CA	Wood 1941
T. protracta	34.0522	-118.2437	Eaton Canyon, Los Angeles County	Wood 1941
T. protracta	32.7098	-108.302	Tyrone, NM	Wood 1941
T. protracta	32.7157	-117.1611	Carroll Canyon, San Diego county	Wood 1941
T. protracta	32.9595	-117.2653	Del Mar, CA	Wood 1941
T. protracta	34.1425	-118.2551	Glendale, CA	Wood 1941
T. protracta	34.175	-117.9871	Monrovia Canyon, Los Angeles County	Wood 1941
T. protracta	34.7166	-118.664	Liebre Mountains, CA	Wood 1941
T. protracta	34.3167	-118.0058	Lower Shake Canyon, CA	Wood 1941

T. protracta	34.1672	-118.4729	Sepulveda Canyon, CA	Wood 1941
T. protracta	33.5849	-116.4568	Pinyon Flats, CA	Wood 1941
T. protracta	34.8697	-111.761	Sedona, AZ	Wood 1941
T. protracta	31.9331	-109.2718	Pinery Canyon, AZ	Wood 1941
T. protracta	32.2084	-109.5759	Dos Cabezas, AZ	Wood 1941
T. protracta	34.19583	-112.7772	Alvardo Mine, AZ	Wood 1941
T. protracta	32.7701	-108.2803	Silver City, NM	Wood 1941
T. protracta	32.7701	-108.2803	Silver City, NM	Wood 1941
T. protracta	37.0475	-112.5263	Kanab, UT	Wood 1941
T. protracta	37.0965	-113.5684	St. George, UT	Wood 1941
T. protracta	29.27	-103.3	Chisos Mountains, TX	Wood 1941
T. protracta	30.2052	-103.2446	Marathon, TX	Wood 1941
T. protracta	28.948	-100.624	Quemado, TX	Wood 1941
T. protracta	33.4484	-112.074	Phoenix, AZ	Wood 1941
T. protracta	34.1625	-112.8507	Congress Junction, AZ	Wood 1941
T. protracta	34.19583	-112.7772	Alvardo Mine, AZ	Wood 1941
T. protracta	32.3199	-106.7637	Las Cruces, NM	Wood 1941
T. protracta	32.4207	-104.2288	Carlsbad, NM	Wood 1941
T. protracta	34.0584	-106.8914	Socorro, NM	Wood 1941
T. protracta	31.562	-106.274	Bosque Bonito, TX	Wood 1941
T. protracta	31.4229	-103.4932	Pecos, TX	Wood 1941
T. protracta	28.7091	-100.4995	Eagle Pass, TX	Wood 1941
T. protracta	27.5306	-99.4803	Laredo, TX	Wood 1941

T. protracta	28.3455	-99.6134	Catarina, TX	Wood 1941
T. protracta	32.7157	-117.1611	San Diego, CA	Kofoid and McCulloch 1916
T. protracta	34.4442	-117.9353	Juniper Hills, CA	Wood 1975
T. sanguisuga	33.9021	-96.3226	Bryan County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.255	-97.3517	Cleveland County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	34.7289	-97.3517	Garvin County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	34.8622	-94.645	Leflore County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.6343	-96.8351	Lincoln County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.2322	-95.3103	Mayes County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.6038	-97.3517	Oklahoma County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.6189	-96.2376	Osage County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.145	-97.0068	Payne County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	34.7685	-96.6638	Pontotoc County, OK	Usinger 1944; Drew and Schaefer 1962

T. sanguisuga	35.2754	-97.0068	Pottawatomie County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.4138	-99.3673	Woodward County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.8825	-98.3965	Blaine County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.9405	-97.5247	Logan County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.8204	-99.6341	Harper County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.4799	-97.179	Noble County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	36.6771	-95.941	Washington County, OK	Usinger 1944; Drew and Schaefer 1962
T. sanguisuga	35.7724	-86.3377	Rutherford County, TN	Herwaldt et al. 2000
T. sanguisuga	31.9973	-102.0779	Midland County, TX	Kjos et al. 2009
T. sanguisuga	31.3839	-100.4397	Tom Green County, TX	Kjos et al. 2009
T. sanguisuga	31.7705	-98.9245	Brown County, TX	Kjos et al. 2009
T. sanguisuga	33.1403	-99.8125	Haskell County, TX	Kjos et al. 2009
T. sanguisuga	33.22	-98.7481	Young County, TX	Kjos et al. 2009
T. sanguisuga	33.5762	-98.7481	Archer County, TX	Kjos et al. 2009
T. sanguisuga	33.931	-98.7481	Wichita County, TX	Kjos et al. 2009
T. sanguisuga	33.79	-98.2213	Clay County, TX	Kjos et al. 2009

T. sanguisuga	32.7732	-97.3517	Tarrant County, TX	Kjos et al. 2009
T. sanguisuga	32.7767	-96.797	Dallas County, TX	Kjos et al. 2009
T. sanguisuga	32.0622	-97.179	Hill County, TX	Kjos et al. 2009
T. sanguisuga	32.1793	-98.2213	Erath County, TX	Kjos et al. 2009
T. sanguisuga	31.8508	-97.6982	Bosque County, TX	Kjos et al. 2009
T. sanguisuga	31.5182	-97.179	Mclennan County, TX	Kjos et al. 2009
T. sanguisuga	31.7769	-95.6458	Anderson County, TX	Kjos et al. 2009
T. sanguisuga	31.6353	-94.645	Nacogdoches County, TX	Kjos et al. 2009
T. sanguisuga	31.0688	-95.1432	Trinity County, TX	Kjos et al. 2009
T. sanguisuga	31.1344	-97.5247	Bell County, TX	Kjos et al. 2009
T. sanguisuga	30.8093	-96.9795	Milam County, TX	Kjos et al. 2009
T. sanguisuga	30.45	-96.6638	Burleson County, TX	Kjos et al. 2009
T. sanguisuga	30.6504	-96.3226	Brazos County, TX	Kjos et al. 2009
T. sanguisuga	30.2967	-96.9639	Lee County, TX	Kjos et al. 2009
T. sanguisuga	29.8896	-96.8351	Fayette County, TX	Kjos et al. 2009
T. sanguisuga	30.727	-98.2213	Burnet County, TX	Kjos et al. 2009
T. sanguisuga	30.0538	-98.0029	Hays County, TX	Kjos et al. 2009
T. sanguisuga	30.2097	-97.6982	Travis County, TX	Kjos et al. 2009
T. sanguisuga	30.7592	-97.6982	Williamson County, TX	Kjos et al. 2009
T. sanguisuga	30.0945	-99.4562	Kerr County, TX	Kjos et al. 2009
T. sanguisuga	29.4201	-98.5721	Bexar County, TX	Kjos et al. 2009
T. sanguisuga	29.4835	-97.5247	Gonzales County, TX	Kjos et al. 2009
T. sanguisuga	29.1235	-97.3517	Dewitt County, TX	Kjos et al. 2009

T. sanguisuga	29.3359	-96.8351	Lavaca County, TX	Kjos et al. 2009
T. sanguisuga	28.7713	-97.0068	Victoria County, TX	Kjos et al. 2009
T. sanguisuga	28.4169	-96.6638	Calhoun County, TX	Kjos et al. 2009
T. sanguisuga	28.8205	-95.9833	Matagorda County, TX	Kjos et al. 2009
T. sanguisuga	29.2132	-95.4778	Brazoria County, TX	Kjos et al. 2009
T. sanguisuga	29.7752	-95.3103	Harris County, TX	Kjos et al. 2009
T. sanguisuga	28.3624	-97.6982	Bee County, TX	Kjos et al. 2009
T. sanguisuga	29.3343	-99.8125	Uvalde County, TX	Kjos et al. 2009
T. sanguisuga	28.8801	-99.7233	Zavala County, TX	Kjos et al. 2009
T. sanguisuga	34.5509	-102.3119	Dimmitt County, TX	Kjos et al. 2009
T. sanguisuga	27.8174	-99.0129	Webb County, TX	Kjos et al. 2009
T. sanguisuga	27.7525	-98.5721	Duval County, TX	Kjos et al. 2009
T. sanguisuga	27.7842	-98.0465	Jim Wells County, TX	Kjos et al. 2009
T. sanguisuga	26.4656	-98.2213	Hidalgo County, TX	Kjos et al. 2009
T. sanguisuga	26.1285	-97.5247	Cameron County, TX	Kjos et al. 2009
T. sanguisuga	32.3182	-86.9023	Alabama	Lent and Wygodzinsky 1979
T. sanguisuga	34.0489	-111.0937	Arizona	Lent and Wygodzinsky 1979
T. sanguisuga	35.201	-91.8318	Arkansas	Lent and Wygodzinsky 1979
T. sanguisuga	27.6648	-81.5158	Florida	Lent and Wygodzinsky 1979
T. sanguisuga	32.1656	-82.9001	Georgia	Lent and Wygodzinsky 1979
T. sanguisuga	40.6331	-89.3985	Illinois	Lent and Wygodzinsky 1979
T. sanguisuga	40.2672	-86.1349	Indiana	Lent and Wygodzinsky 1979
T. sanguisuga	39.0119	-98.4842	Kansas	Lent and Wygodzinsky 1979

T. sanguisuga	37.8393	-84.27	Kentucky	Lent and Wygodzinsky 1979
T. sanguisuga	30.9843	-91.9623	Louisiana	Lent and Wygodzinsky 1979
T. sanguisuga	39.0458	-76.6413	Maryland	Lent and Wygodzinsky 1979
T. sanguisuga	32.3547	-89.3985	Mississippi	Lent and Wygodzinsky 1979
T. sanguisuga	37.9643	-91.8318	Missouri	Lent and Wygodzinsky 1979
T. sanguisuga	35.7596	-79.0193	North Carolina	Lent and Wygodzinsky 1979
T. sanguisuga	40.4173	-82.9071	Ohio	Lent and Wygodzinsky 1979
T. sanguisuga	35.0078	-97.0929	Oklahoma	Lent and Wygodzinsky 1979
T. sanguisuga	41.2033	-77.1945	Pennsylvania	Lent and Wygodzinsky 1979
T. sanguisuga	33.8361	-81.1637	South Carolina	Lent and Wygodzinsky 1979
T. sanguisuga	35.5175	-86.5804	Tennessee	Lent and Wygodzinsky 1979
T. sanguisuga	37.4316	-78.6569	Virgina	Lent and Wygodzinsky 1979
T. sanguisuga	30.0459	-97.3517	Bastrop County, TX	Sarkar et al. 2010
T. sanguisuga	32.5889	-96.3089	Kaufman County, TX	Sarkar et al. 2010
T. sanguisuga	28.8205	-95.9833	Matagorda County, TX	Sullivan et al. 1949
T. sanguisuga	27.5989	-98.4081	Benavides, TX	Wood 1941
T. sanguisuga	27.4448	-98.5283	Realitos, TX	Wood 1941
T. sanguisuga	27.4486	-99.0873	Aguilares, TX	Wood 1941
T. sanguisuga	30.8533	-81.4389	Cumberland Island, Georgia	Roden et al. 2011
T. sanguisuga	31.4764	-81.2409	Sapelo Island, Georgia	Roden et al. 2011
T. sanguisuga	29.6516	-82.3248	Gainesville, FL	Beard et al. 1988
T. sanguisuga	29.9511	-90.0715	New Orleans, LA	Dorn et al. 2007

T. sanguisuga	35.4676	-97.5164	Oklahoma County, OK	Griffith 1948
T. sanguisuga	39.1836	-96.5717	Manhattan, Kansas	Grundemann 1947
T. sanguisuga	32.3596	-81.7787	Bulloch County, GA	Pung et al. 1995
T. sanguisuga	29.658	-82.3018	Alachua County, FL	Thurman 1948
T. sanguisuga	29.5207	-83.1649	Dixie County, FL	Thurman 1948
T. sanguisuga	30.3501	-81.6035	Duval County, FL	Thurman 1948
T. sanguisuga	29.6871	-82.821	Gilchrist County, FL	Thurman 1948
T. sanguisuga	26.6105	-81.0755	Hendry County, FL	Thurman 1948
T. sanguisuga	30.7151	-85.1894	Jackson County, FL	Thurman 1948
T. sanguisuga	30.03	-83.2078	Lafayette County, FL	Thurman 1948
T. sanguisuga	30.4906	-84.1857	Leon County, FL	Thurman 1948
T. sanguisuga	29.3179	-82.821	Levy County, FL	Thurman 1948
T. sanguisuga	30.4586	-83.507	Madison County, FL	Thurman 1948
T. sanguisuga	29.2788	-82.1278	Marion County, FL	Thurman 1948
T. sanguisuga	28.3232	-82.4319	Pasco County, FL	Thurman 1948
T. sanguisuga	28.6748	-82.0843	Sumter County, FL	Thurman 1948
T. sanguisuga	30.2485	-82.9932	Suwannee County, FL	Thurman 1948
T. sanguisuga	30.0994	-83.6774	Taylor County, FL	Thurman 1948
T. sanguisuga	29.9719	-81.4279	St. Johns County, FL	Thurman 1948
T. sanguisuga	28.8849	-82.5186	Citrus County, FL	Thurman 1948
T. sanguisuga	34.9046	-82.6483	Pickens County, SC	Yabsley and Noblet 2002
T. sanguisuga	34.5528	-82.6483	Anderson County, SC	Yabsley and Noblet 2002
T. sanguisuga	34.6613	-81.6035	Union County, SC	Yabsley and Noblet 2002

T. sanguisuga	34.0795	-82.8641	Elbert County, GA	Yabsley and Noblet 2002
T. sanguisuga	33.8083	-82.7779	Wilkes County, GA	Yabsley and Noblet 2002
T. sanguisuga	33.9519	-83.3576	Clarke County, GA	Yabsley and Noblet 2002
T. sanguisuga	33.912	-83.0361	Oglethorpe County, GA	Yabsley and Noblet 2002
T. sanguisuga	33.7875	-82.4319	Lincoln County, GA	Yabsley and Noblet 2002
T. sanguisuga	34.0412	-80.9429	Richland County, SC	Yabsley and Noblet 2002
T. sanguisuga	33.6739	-80.7658	Calhoun County, SC	Yabsley and Noblet 2002
T. sanguisuga	33.6006	-81.6035	Aiken County, SC	Yabsley and Noblet 2002
T. sanguisuga	32.4914	-81.0755	Jasper County, SC	Yabsley and Noblet 2002
T. sanguisuga	32.8085	-81.1196	Hampton County, SC	Yabsley and Noblet 2002
T. sanguisuga	32.7957	-79.7848	Charleston County, SC	Yabsley and Noblet 2002
T. sanguisuga	33.9197	-78.9288	Horry County, SC	Yabsley and Noblet 2002

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