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Population Genetics Among *Rhipicephalus sanguineus* Ticks in the Lower Rio Grande Valley

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POPULATION GENETICS AMONG RHIPICEPHALUS SANGUINEUS TICKS IN THE LOWER RIO GRANDE VALLEY

A Thesis

by

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ABSTRACT

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Rhipicephalus sanguineus is a common tick species in the lower Rio Grande Valley of both human and veterinary concern. Two lineages of *R. sanguineus* have been described across the United States known as the tropical and temperate lineage. Both lineages can be differentiated morphologically, genetically, biologically, and by vector competence. The species name for both lineages has not been well established therefore this thesis will utilize their widely used identification. Both lineages have been identified in Texas. While the distribution of these lineages has been well-defined in some regions across the world, recent studies have observed sympatric populations in certain areas, suggesting a need for further investigation into their distribution and potential overlap. The aim of this study is to explore the genetic variation among different populations in the lower Rio Grande Valley by conducting a sequence analysis of the 12S rRNA mitochondrial gene. The presence of one or both lineages in the Rio Grande Valley may help predict the relative risk of some tick-borne diseases. Collections were conducted weekly for five months. A sample size of 250 *R. sanguineus* ticks were utilized in this study. The sequence analysis of 12S rRNA indicated only the presence of the tropical lineage, confirming previous studies that only found that lineage in this region.

DEDICATION

I dedicate this thesis and the completion of my Master of Science degree in Biology to my mother Esmeralda Guerra and Father Alfredo Guerra. Thank you both for raising me to always strive for success and being my number one support system. I also want to dedicate this thesis to my friends and family who have always encouraged me to persevere in anything I do.

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CHAPTER I

INTRODUCTION

Ticks are important vectors within the Arthropoda phylum, responsible for the maintenance and transmission of various pathogens including bacteria, helminths, protozoa, and viruses that affect both humans and animals (Oliver, 1989; Dantas-Torres, 2010; Jongejan and Uilenberg, 2004; Parola et al., 2009). One commonly known tick species is *Rhipicephalus sanguineus,* also referred to as the brown dog tick due to its preference for domestic dogs as hosts. *R. sanguineus* is of both human and veterinary importance as it transmits several human diseases, such as *Rickettsia rickettsia*, *Ehrlichia chaffeensis*, and *Rickettsia conorii* (Parker et al., 1933; Brumpt, 1932; Matsumoto et al., 2005; Parola et al., 2009; Stoffel et al., 2014), and diseases of veterinary importance such as *Babesia gibsoni*, *Babesia canis*, *Ehrlichia canis*, and *Hepatozoon canis* (Sen, 1933; Regendanz and Muniz, 1936; Groves et al., 1975; Nordgren and Craig, 1984; Demoner, 2013). This external parasite affects the livestock industry, companion canines, and human populations across the globe (Dantas-Torres, 2008; Parola et al., 2009; Vasquez et al., 2019).

The taxonomic status of *Rhipicephalus sanguineus* has been a subject of continuous debate and discussion (Feldman-Muhsam, 1952; Pegram et al., 1987; Zahler et al., 1997; Barker, 1998; Matsumoto et al., 2005; Szabo et al., 2005; Dantas-Torres, 2008; Nava et al., 2015). Originally, Latreille (1806) identified *R. sanguineus* as *Ixodes sanguineus* based on a population

in France. However, the description of the tick was brief and vague. Later, Koch (1844) transferred the taxonomic position of this tick to the *Rhipicephalus genus* based on populations in Portugal. Both descriptions were based solely on morphological characteristics with no mention of origin, specific behavior, or geographic distribution. Previous studies have indicated that different populations identified as *Rhipicephalus sanguineus* based on classical morphological descriptions exhibit genetic and biological differences, including morphological characteristics, climate and host preference, and geographic isolation (Cooley, 1946; Pegram et al., 1987; Filippova, 1997; Walker et al., 2000; Oliveira et al., 2005; Jones et al., 2017; Grant et al., 2023). This suggests that there may be multiple lineages, potentially different species, all identified as *R. sanguineus*.

Numerous studies have documented the existence of distinct populations of *Rhipicephalus sanguineus* ticks across the globe, including Australia, Brazil, Colombia, Costa Rica, France, Guatemala, Honduras, India, South Africa, Thailand, and Vietnam (Szabo et al., 2005; Oliveira, 2005; Burlini et al., 2010; Moraes-Filho et al., 2011; Levin et al., 2012; Dantas-Torres, 2013). These investigations have established that *R. sanguineus* is comprised of two genetically and morphologically divergent lineages known as the tropical and temperate lineages. Based on behavioral, genetic, and morphological distinctions, the tropical and temperate lineages have been classified as distinct species by Slapeta and Chandra (2021), Brophy (2022), and Grant et al. (2023). The tropical lineage is commonly referred to as *Rhipicephalus sanguineus* sensu lato (s.l) (Audouin, 1826; Nava et al., 2018) and has been reclassified as *Rhipicephalus linnaei* by Slapeta and Chandra (2021). In contrast, the temperate lineage, has been tentatively referred to as *Rhipicephalus* sensu stricto (s.s.) and *Rhipicephalus* sp. II in previous studies (Moraes-Filho et al., 2011; Nava et al., 2018; Šlapeta et al., 2021; Šlapeta, 2022). However, this

classification does not represent an official taxonomic designation, as the temperate lineage has not yet been assigned a formal species name. For consistency with prior studies, both lineages will be referred to as the tropical and temperate lineages in this thesis (Szabo et al., 2005; Dantas-Torres, 2008; Levin et al., 2012; Dantas-Torres et al., 2013; Dantas-Torres and Otranto, 2015; Nava et al., 2015; Labruna et al., 2017; Villarreal et al., 2018; Backus et al., 2021). The focus of this thesis is on the population genetics dynamics of both the tropical and temperate lineages of *Rhipicephalus sanguineus*.

Biology and Ecology

The well-recognized brown dog tick can be found across the world in both urban and rural areas. *Rhipicephalus sanguineu*s is known for its reddish-brown color and its preferred host, the domestic dog. This tick can adapt to different environmental settings such as human dwellings and tropical and temperate regions (Koch, 1982; Dantas-Torres, 2008; Dantas-Torres, 2010). *R. sanguineus* can be classified as endophilic (adapted to indoor environments) and monotrophic (all developmental stages feed on the same host species). This tick can survive in various outdoor environments and refuges such as the crevices of walls and outdoor dog shelters (Koch and Tuck, 1986; Dantas-Torres, 2008; Dantas-Torres, 2010;). *R. sanguineus* life cycle consists of four developmental stages known as egg, larvae, nymph, and adult (Figure 2A). Of these developmental stages, blood meals will be consumed in the larva, nymph, and adult stage, classifying *R. sanguineus* as a three-host tick (Figure 2A) (Dantas-Torres, 2010). This species can be differentiated by sex, feeding behaviors, and developmental life stage.

Female and male *R. sanguineus* adults can be identified by specific morphological traits. The morphology of adult males has been characterized as flat (2.28–3.18 mm long by 1.11–1.68 mm wide) and reddish-brown with tiny pits scattered over the back (Dantas-Torres 2008). Adult females will initially resemble the males in size, color, and shape. However, engorged females can swell up to approximately 11.5 mm long by 7.5 mm wide (Dantas-Torres, 2008). The enlarged portion of the engorged female will become a gray blue color. Both adults share a similar darkened feature called the scutum or often referred to as a shield. The female adults will have a smaller scutum on the anterior dorsal surface while the males scutum will cover its entire dorsal area (Figure 1A).

When seeking a host, *Rhipicephalus sanguineus* adopts the host-seeking behavior known as questing. This ambush strategy has been acquired throughout its evolutionary history from its relationship with the canine and shared environment (Dantas-Torres, 2010). Once the tick latches onto its host, it utilizes its chelicerae and hypostome to penetrate through the epidermis of the host skin and secretes a cement-like substance that forms a cone on the surface of the epidermis (Szabo and Bechara, 1999; Dantas-Torres 2010). *R. sanguineus* can attach anywhere on the canine hosts body with a preference for the head, ears, back, inguinal region, and axilla area (Koch, 1982). When probing for blood, the capillary and blood vessels will be lacerated, and hemorrhaging will occur (Dantas-Torres, 2010). This will create a blood pool, called a feeding pool, allowing the tick to intake blood and other fluids (Man's, 2004). *R. sanguineus* will then enter its feeding period that can last a duration of five to twenty-one days (Gray et al., 2013). Male adults can also attach for several weeks; however, they will often consume smaller blood meals and stop feeding earlier than females (Koch, 1982; Gray and Dantas-Torres, 2008).

Adult *Rhipicephalus sanguineus* ticks not only rely on their host for blood meals, but also for sexual mating. Both male and female ticks consume multiple blood meals for development and reproduction (Hadi and Adventini, 2015). Females require large quantities of blood to stimulate oogenesis and produce eggs, which can increase their body weight by a factor of 100 (Szabó, 2005). Males consume less blood than females, but still need it for spermatogenesis during mating (Szabó, 2005; Dantas-Torres, 2010). Mating typically occurs when the male climbs onto the female's dorsal side, then positions himself center-to-center with her and stimulates her gonopore by inserting the tip of his chelicerae, which allows him to transfer the spermatophore to the genital aperture (Feldman-Muhsam and Borut, 1971). The spermatophore enters the female's genital tract, and fertilization commences (Feldman-Muhsam and Borut, 1971). After mating, adult ticks detach from the host or are "dropped-off," a behavior that is often coordinated with the host's activity (Oliver, 1989). Males typically die after mating (Koch, 1982; Dantas-Torres, 2010).

After the engorged female detaches from its host it will seek refuge and enter a pre-oviposition period. The duration of this period can vary from three days to a couple of weeks (Dantas-Torres, 2010). After the pre-oviposition period, the female will then enter its oviposition period and deposit approximately 1500-4000 eggs (Koch, 1982; Dantas-Torres, 2010). The oviposition period can have a timespan of several weeks. The duration of the oviposition period and amount of blood ingested will directly correspond with the number of eggs that will be produced (Koch, 1982; Oliver et al., 1989; Dantas-Torres, 2010). These eggs are approximately 0.5 mm length and 0.4 mm width, spherical with a dark brown color, and have an average egg mass weight of 32.68 mg/tick (5-70 mg/tick) (Hadi and Adventini, 2015). The eggs will be laid in hidden places or refuges such as cracks and crevices in the walls that will provide

the female and her eggs protection from predators such as spiders, birds, and wasps (Dantas-Torres et al., 2006; Dantas-Torres, 2010).

The hatching of an egg marks the beginning of the *Rhipicephalus sanguineus* larval stage, which lasts from 6 days to several weeks before its exoskeleton hardens and it becomes capable of seeking out a host (Dantas-Torres, 2008; Dantas-Torres, 2010; Hadi and Adventini, 2015). Unlike the more developed stages, larvae have only three pairs of legs (Dantas-Torres, 2008). Upon finding a host, the larva will feed on blood for a period of 2-15 days depending on environmental conditions, such as temperature (Ioffe-Uspensky et al., 1997; Hadi et al., 2015). Once satiated, the larva will detach from the host, with diurnal drop-off patterns observed mainly during the day (Paz, 2008). It then seeks shelter and undergoes molting to transition to its next life stage, a process that may take days to weeks depending on various stressors, including weather conditions (Dantas-Torres, 2010).

After molting, the larvae will then enter its nymph stage. Like its larvae stage, the nymph will acquire a host and blood feed. Nymphs will have a feeding period between three to six days (Koch, 1982; Gray and Dantas-Torres, 2008). It will then detach from its host and seek refuge where it will enter its molting period. The molting period for nymphs is much longer than in larvae, however, if exposed to stressful temperatures and humidity then the molting period can be extended (Dantas-Torres, 2010). Low temperatures cause the larvae and nymphs to undergo diapause while high temperatures will shorten the molting period (Koch, 1986). Once the molting is completed, the nymph will then become an adult tick and the new generation of ticks will fulfill its life cycle. The overall life cycle from egg to egg-laying female can be completed in two months (Louly et al., 2007). However, overall development will frequently be prolonged due

to low host availability and freezing temperatures (Louly et al., 2007). *R. sanguineus* can survive without three to five months without blood feeding between each life stage (Tian et al., 2020).

Pathogens and Hosts

Rhipicephalus sanguineus is a common vector for various human and canine diseases, serving as a reservoir for pathogens such as *Rickettsia rickettsii, Rickettsia conorii, Ehrlichia chaffeensis, Babesia gibsoni, Babesia canis, Ehrlichia canis,* and *Hepatozoon canis* (Parker et al., 1933; Sen, 1933; Brumpt, 1932; Regendanz and Muniz, 1936; Groves et al., 1975; Nordgren and Craig, 1984; Matsumoto et al., 2005; Parola et al., 2009; Demoner, 2013). While most of these pathogens affect canines, some can also infect humans (Dantas-Torres, 2007; Dantas-Torres and Otranto, 2015). *R. sanguineus* is a cosmopolitan species, likely due to the ubiquitous presence of its preferred host, the domestic dog. As such, these ticks are commonly found in kennels and residential areas. Although *R. sanguineus* can infest a variety of hosts, both domestic and wild, such as cats, rodents, dogs, birds, and humans (Lori, 1996; Hadi and Adventini, 2015), instances of infestations on animals other than dogs and humans are rare and may depend on tick population and environment (Demma et al., 2005; Dantas-Torres, 2007; Backus et al., 2021). Infestations of *R. sanguineus* are most found on canines and shared environments (Hadi and Adventini, 2015; Dantas-Torres, 2009). The preference of *R. sanguineus* between humans and canines is influenced by climate and locality (Parola, 2008; Backus et al., 2021). Previous studies have shown that *R. sanguineus* populations in South America more commonly attach to humans than those in North America (Dantas-Torres, 2007; Guglielmone, 2006; Mentz et al., 2016). However, human host preference may differ by region in different countries (Dantas-Torres, 2010; Mentz et al., 2016; Backus, 2021).

Rhipicephalus sanguineus is known to transmit various pathogens belonging to the Ehrlichiosis group, including *Ehrlichia canis, Ehrlichia ewingii,* and *Ehrlichia chaffeensis* (Aziz et al., 2022). Ehrlichiosis is a gram-negative bacterium that is vectored by ticks and responsible for multiple diseases (Moraes-Filho et al., 2015). This group is most prevalent in tropical and subtropical areas (Aziz et al., 2022). In canines, Ehrlichiosis is recognized as canine rickettsiosis, canine typhus, canine hemorrhagic fever, tropical canine pancytopenia, and tracker dog disease (Beall et al., 2012). *E. canis* is a common pathogen in canines responsible for canine ehrlichiosis, which affects platelets, monocytes, and granulocytes (Stiles, 2000; Moraes-Filho et al., 2015). This disease is well known for causing the death of many military German Shepherd canines during the Vietnam War in the 1970s (Amyx et al., 1971). Komnenou et al. (2007) investigated the symptoms of *E. canis* in canines and observed high fever, anorexia, lethargy, lymphadenomegaly, depression, epistaxis, splenomegaly, petechial, and ecchymotic skin hemorrhages. This pathogen can be found throughout the United States and is frequently diagnosed in dogs living in the southeastern and southwestern states (Skyes, 2014). *E. chaffeensis* is the main pathogen responsible for human monocytic ehrlichiosis (HME) and is often reported in canines (Aziz et al., 2022). Although *E. chaffeensis* is commonly transmitted by *Amblyomma americanium*, studies have determined *R. sanguineus* as a vector for this pathogen (Ndip et al., 2007; Ndip et al., 2010; Aziz et al., 2022). *E. chaffeensis* is the etiologic agent of human monocytotropic ehrlichiosis (HME) with common symptoms of fever, headache, hematologic abnormalities, and elevated liver enzymes (Ismail and McBride, 2017). The Centers for Disease Control and Prevention (CDC) reported an increase in HME from 1 per million to 3.4 per million from the year 2000 to 2010 (Demma et al., 2005). However, a prospective study

conducted in endemic areas proposed a rate of 100 to 200 cases per million based on active surveillance for three years in family physicians' practice (Walker, 2005).

Another common pathogenic group vectored by *Rhipicephalus sanguineus* is referred to as the spotted fever group including *Rickettsia conorii* and *Rickettsia rickettsii* (Kordick et al., 1999; Matsumoto et al., 2005; Dantas-Torres, 2007; Grays and Dantas-Torres, 2013; Dantas-Torres and Otranto, 2015). *Rickettsia conorii* is the causative agent for the Mediterranean spotted fever and a few types of tick typhus (Parola et al. 2009; Zemtsova et al. 2010). This disease has been reported in various parts of Europe, Asia, and Africa (Zemstova et al., 2010). The most common and severe rickettsial infection is *Rickettsia rickettsii* known as the disease Rocky Mountain spotted fever (RMSF) (Snowden et al., 2023). Symptoms of this disease include fever, headache, rash, nausea, stomach pain, muscle pain, and lack of appetite and can be treated with antibiotic (Dantas-Torres, 2007). Without prompt treatment, RMSF can have a mortality rate as high as 20 to 30 percent (Snowden et al., 2023). This disease is most commonly prevalent in regions of Arizona and Californian with low infection reports in other states (Parker et al., 1933; Demma et al. 2005; Wikswo et al. 2007). In Mexico, Rocky Mountain spotted fever has been an epidemiological emergence, with high case fatality rates (Alvarez-Hernandez et al. 2008; Straily, 2016).

Pathogenic transmission can be influenced by various factors such as climate and attachment rate (Koch, 1982; Gray et al., 2009; Backus et al., 2021). Climate change can directly impact the epidemiology of tick-borne diseases. Research has shown that high temperatures can increase tick attachment and feeding on humans, which suggests that areas experiencing extended periods of warmer climates may have an increased risk of zoonotic agent infections (Gray et al., 2009; Dantas-Torres, 2010; Backus et al., 2021). Additionally, studies have found that higher ambient

temperatures and lower humidity can lead to more aggressive tick behavior towards human hosts (Parola, 2008; Backus, 2021), with reports of increased Rickettsial disease and human feeding seen during heat waves (Parola, 2008; Backus, 2021). This suggests that areas experiencing warmer climates could potentially increase the risk of disease transmission and provoke questing behavior. In canines, studies have indicated that breed can influence host attachment. For example, Louly (2009) found that *R. sanguineus* infestation varied between different canine breeds, with English Crocker spaniels being ten times more infested than mongrels in Brazil. Louly (2010) then investigated whether canine odor influences *R. sanguineus* preference based on English Crocker spaniels and beagles. The study found that 57% of the ticks were attracted to English Crocker spaniels while 43% were attracted to beagles. As humans and canines often share environments, humans are at an increased risk of tick encounters and attachment. Further research is necessary to determine which canine breeds are more susceptible to *R. sangineus* infestation than others, and to explore odor preferences of *R. sanguineus* for different dog breeds and humans.

Tropical and Temperate Lineage

Rhipicephalus sanguineus was originally named *Ixodes sanguineus* by Latreille (1806) based on a population in France. Latreille described the ticks as "blood red, punctate, posteriorly with three impressed lines; no distinct 'thoracic' spot anterodorsally (Dantas-Torres et al., 2013). Although Latreilles description was acceptable during the 1800's, it is a vague and poor description that cannot be utilized in the present day. The taxonomic position of this tick was then reclassified to the *Rhipicephalus* genus by Koch (1844) based on populations in Portugal. The descriptions of this species were based on morphological characteristics with no mention of origin or specific behavior and geographic distribution. Over time, several studies have indicated that different populations identified as *Rhipicephalus sanguineus*, following the classical morphological descriptions, showed both genetic and biological differences including feeding behavior, temperature preference, and geographic isolation (Cooley, 1946; Filippova, 1997, Pegram et al., 1987, Walker et al., 2000; Oliveira et al., 2005; Jones et al., 2017; Grant et al., 2023). The classical descriptions of *R. sanguineus* was not well detailed therefore different species under the *Rhipicephalus* genus have often been identified as *R. sanguineus* (Pegram et al., 1987; Farid, 1997; Nava et al., 2015). Strict morphological identification and genetic and biological criteria are needed to ensure accurate identification. Camicas and colleagues (1998) have determined there are 17 different species within the *R. sanguineus* group including *Rhipicephalus aurantiacus, Rhipicephalus guilhoni, Rhipicephalus leporis, Rhipicephalus moucheti, Rhipicephalus pumilio, Rhipicephalus pusillus, Rhipicephalus turanicus, and Rhipicephalus ziemanni*. Some of these species can be identified using an updated dichotomous key by Walker et al. (2023) conducted in Scotland. These species are often

collectively referred to *as Rhipicephalus sanguineus* or *Rhiphicephalus* sensu lato meaning "in broad sense" due to historical and taxonomic reasons.

Recent genetic studies have revealed that *Rhipicephalus sanguineus* populations from different regions are genetically distinct and can be grouped into two lineages: the temperate lineage and the tropical lineage (Szabo et al., 2005; Dantas-Torres, 2008; Burlini et al., 2010; Moraes-Filho et al., 2011; Levin et al., 2012; Nava et al., 2012; Dantas-Torres, 2013; Labruna et al., 2017; Dantas-Torres et al., 2018). The temperate lineage includes tick populations from Argentina, Uruguay, Chile, and Italy, while the tropical lineage includes ticks from Brazil, Paraguay, Colombia, South Africa, Mozambique, and two locations in Northern Argentina. (Nava et al., 2012). Dantas-Torres (2013) further investigated the morphological and genetic differences of *R. sanguineus* ticks from Europe, Asian, Africa, the Americas, and Oceania (Australia) as a continuation of the studies previously mentioned. The results of this study concluded there is irrefutable evidence that these two lineages are different species under the name *R. sanguineus*. These findings have been supported by subsequent studies conducted by Nava et al. (2018), Tucker et al. (2020), Bakkes et al. (2020), Slapeta et al. (2021), and Walker et al. (2022). Slapeta and Chandra (2021) recently reclassified the tropical lineage as *Rhipicephalus linnaei* based on morphological characteristics described by Audouin, (1826) and genetic analysis using *cox1*, 12s rDNA, and 16S rDNA using *R. sanguineus* populations from Australia.

Morphologically, it was observed that both lineages are distinguishable in size, number of setae on the anal valves, mean size of chelicerae, size and distribution of chitinous plates, and festoon morphology (Oliveria, 2005; Szabó et al., 2005; Dantas-Torres, 2013). However, both lineages were noted to share similar features including grooves and anal aperture (Oliveria, 2005). Aside from morphological differences, both lineages can be distinguished by biological

factors including feeding and developmental behaviors. Szabo et al. (2005) conducted a study in South America to distinguish two dissimilar populations of *Rhipicephalus sanguineus* from Brazil, the tropical lineage, and Argentina, the temperate lineage. The engorged larvae, nymphs, and adults collected from Argentina where morphologically characterized as being heavier with a longer engorgement period by 50% in comparison to Brazil. The two populations were then cross mated, and a non-fertile female was produced. Other studies have reported distinct climate preferences between two lineages (Parola et al., 2009; Dantas-Torres, 2015; Backus, 2021). The tropical lineage can be found in regions with an annual temperature of exceeding 20°C, while the temperate lineage is in regions with an annual temperature 20°C or below (Jones et al., 2017; Zemtsova, 2010; Backus, 2021). Backus (2021) explored how temperature influences host preference between tropical and temperate tick populations. The results indicated that the tropical lineage adults were 2.5 times more likely to migrate towards the human host at a higher temperature. The temperate lineage had a 66% reduction in preference for canine in high temperatures and a greater preference for humans at room temperature. The results of this study suggest that host choice and feeding behavior are influenced by the interaction between lineage and temperature.

Studies have shown that there are differences in the vectorial capacity between the tropical and temperate lineages, which can vary depending on the pathogen in question (Dantas-Torres, 2010; Alvarez-Hernandez, 2017; Villarreal, 2018; Backus, 2021). For instance, a 2009 study by Dantas-Torres et al. (2012) investigated a canine hepatozoonosis outbreak in southern Italy and found that the ticks involved belonged to the temperate lineage, which was able to transmit *Hepatozoon canis*. In contrast, research conducted in Brazil demonstrated that the tropical lineage of the brown dog tick could not act as a vector for *Hepatozoon canis* (Demoner,

2013). These findings were later supported by studies by Latrofa (2014) and Dantas-Torres et al. (2015), which also determined that the tropical lineage was not a vector for *Hepatozoon canis* while the temperate lineage was. Although both lineages can act as reservoirs for *Rickettsia rickettsii*, which causes Rocky Mountain spotted fever, outbreaks have been linked to each lineage in different regions. In 2004, the temperate lineage was responsible for an outbreak in Arizona (Demma, 2005), while the tropical lineage has been implicated in ongoing outbreaks in Baja, California and Sonora, Mexico (Alvarez-Hernandez, 2017; Villarreal et al., 2018).

The tropical and temperate lineages are geographically separated with no overlap (Eremeeva et al., 2011; Zemtsova et al., 2016; Jones et al., 2017; Hornok et al., 2017; Villarreal et al., 2018; Sanches-Montes, 2021). However, recent studies have found both the temperate and tropical groups living in sympatry (Jones et al., 2017; Rene-Martellet et al., 2017; Arizona Department of Health Services and Disease Control, 2021; Grant et al., 2023). Grant et al. (2023) investigated the geographic distribution of the tropical and temperate lineages in the United States based on collections from canines. The temperate lineage was found in several states, including Arkansas, California, Colorado, Idaho, Illinois, Indiana, Kentucky, North Carolina, New Mexico, Ohio, Oklahoma, Oregon, Tennessee, Utah, Washington, and Wisconsin. Meanwhile, the tropical lineage was present in Florida, Hawaii, Michigan, Minnesota, Nevada, New York, Oklahoma, and South Carolina. Texas and Arizona had mixed infestations on canines; however, it's possible that travel of pets led to a short-lived introduction of these two species within the same premises. The tropical lineage was found in the northern states of Michigan and Minnesota, where the cooler climate was likely due to the travel of dogs with their owners. However, specific cities or regions where the specimens were obtained in each state were not specified, so further research is needed to conclude whether most of the tropical and

temperate lineages are geographically isolated by state or by northern and southern regions with limited sympatric populations. Similarly, Jones et al. (2017) identified the tropical and temperate lineages in different localities across the United States and yielded the same results in the states of Arizona, California, Florida, Hawaii, Illinois, Oklahoma, and Texas. In Texas, the temperate lineage was found in Dallas, El Paso, Fort Worth, and San Angelo, while the tropical lineage was found in Edinburg. Further research is needed to determine whether the temperate lineage is geographically isolated to north Texas or if it is present in south Texas due to travel of companion canines.

Conclusion

Rhipicephalus sanguineus is one of the most common tick species found worldwide and is a vector for multiple pathogens, making it a concern for both human and veterinary medicine. Within the *R. sanguineus* species, there are multiple related species, such as *Rhipicephalus turanicus, Rhipicephalus bergeoni, and Rhipicephalus leporis* (Dantas-Torres, 2010; Walker, 2000). The taxonomic status of this species has been under constant debate due to the vague and brief original description of *R. sanguineus* (Feldman-Muhsam, 1952; Pegram et al., 1987; Zahler et al., 1997; Barker, 1998; Matsumoto et al., 2005; Szabo et al., 2005; Dantas-Torres, 2008; Nava et al., 2015). The tropical and temperate species can be differentiated genetically, morphologically, biologically, and by their vectorial capacity, therefore classifying them as two different species (Oliveira et al., 2005; Szabó et al., 2005; Dantas-Torres et al., 2013; Sanches et al., 2016). Previous studies have indicated that both lineages are geographically isolated. However, new research has shown instances where both lineages are present in the same area (Eremeeva et al., 2011; Zemtsova et al., 2016; Jones et al., 2017; Hornok et al., 2017; Villarreal et al., 2018; Sanches-Montes, 2021; Grant et al., 2023). Further investigation is needed to determine the lineage distribution in Texas.

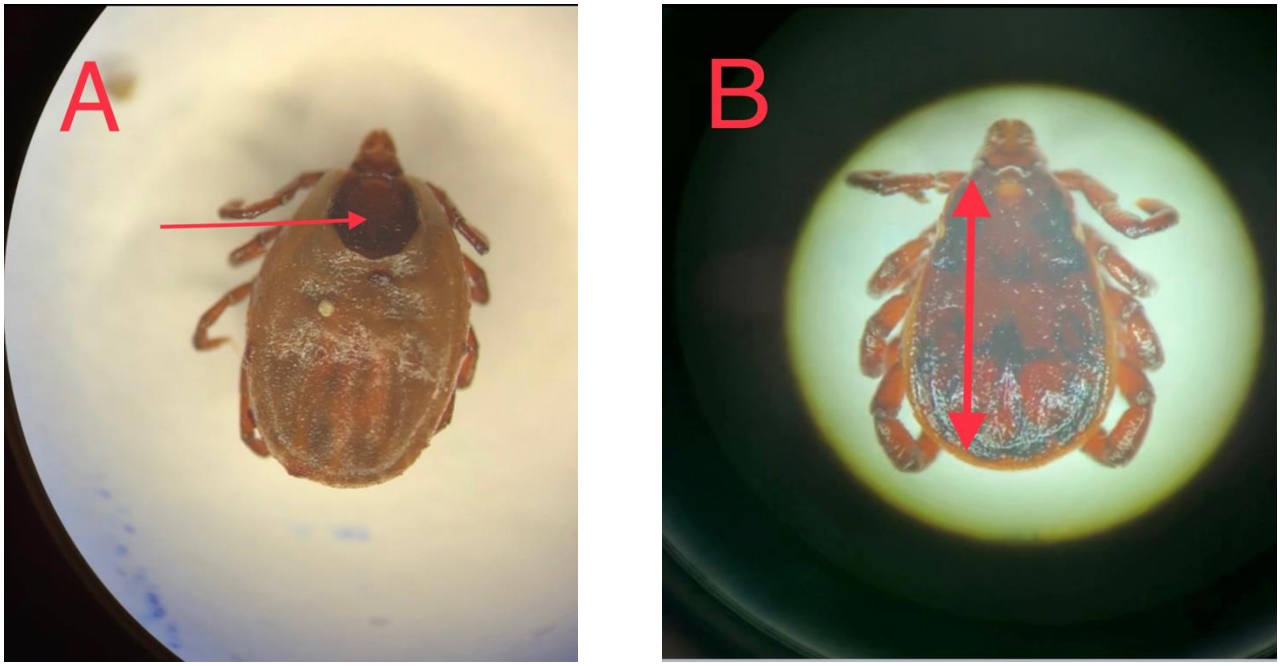


Figure 1**A.** Illustrations depict an engorged adult female and an adult male brown dog tick. Photo A presents the scutum or shield on the female tick with a red arrow. Photo B displays the male tick with a red arrow depicting the scutum that covers its entire dorsal area covering its entire abdomen.

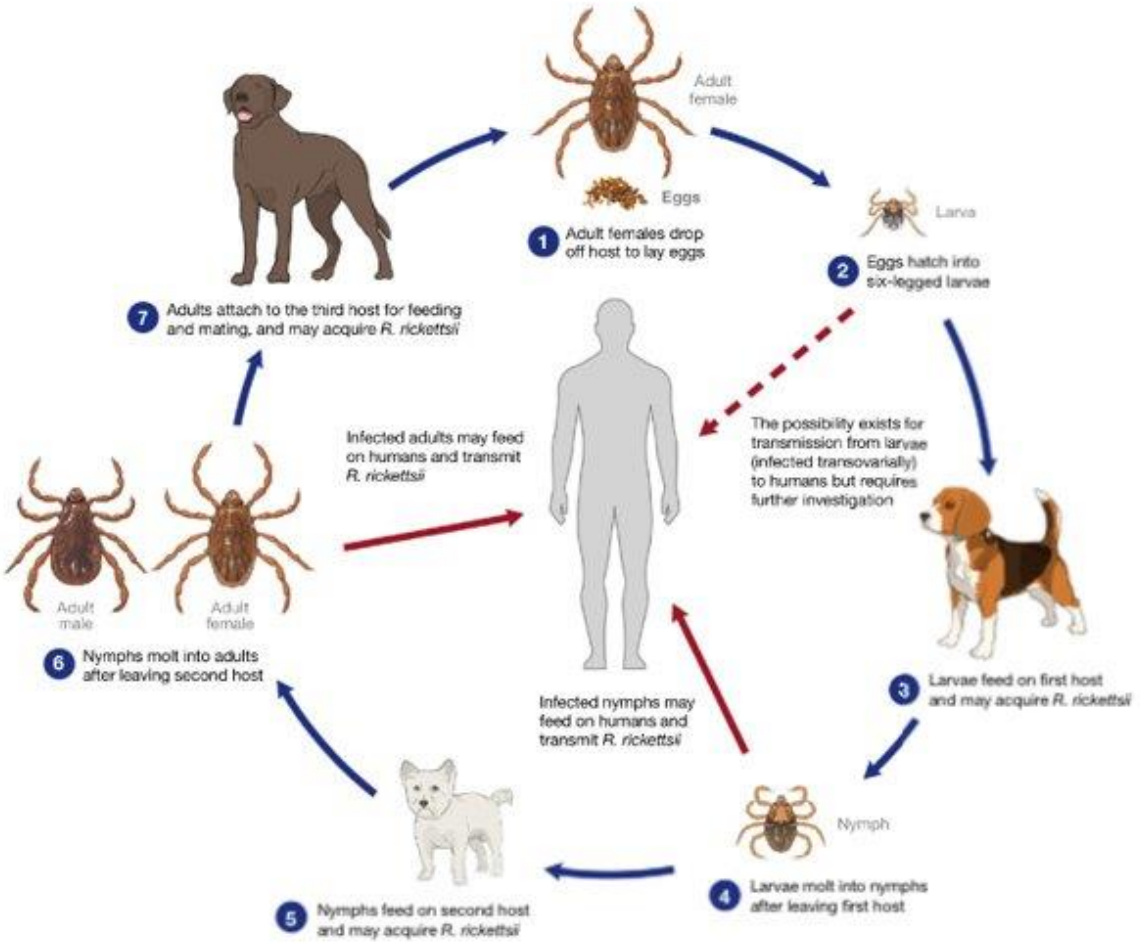


Figure 2A. Illistration of *Rhipicephalus sanguineus* life cycle. This illustration is described by the Centers for Disease Control and Prevention (CDC) (n.d.). The image is used for reference purposes only and was not created by the authors of this study.

CHAPTER II

EXPERIMENT I

Abstract

Rhipicephalus sanguineus is a common tick species in the lower Rio Grande Valley of both human and veterinary concern. Two lineages of *R. sanguineus* have been described across the United States known as the tropical and temperate lineage. Both lineages can be differentiated morphologically, genetically, biologically, and by vector competence. The species name for both lineages has not been well established therefore this thesis will utilize their widely used identification. Both lineages have been identified in Texas. While the distribution of these lineages has been well-defined in some regions across the world, recent studies have observed sympatric populations in certain areas, suggesting a need for further investigation into their distribution and potential overlap. The aim of this study is to explore the genetic variation among different populations in the lower Rio Grande Valley by conducting a sequence analysis of the 12S rRNA mitochondrial gene. The presence of one or both lineages in the Rio Grande Valley may help predict the relative risk of some tick-borne diseases. Collections were conducted weekly for five months, using a variety of field collection methods. A sample size of 250 *R. sanguineus* ticks were utilized in this study. The sequence analysis of 12s rRNA indicated only the presences of the tropical lineage, supporting previous studies.

Introduction

Rhipicephalus sanguineus, also known as the brown dog tick, is a cosmopolitan species of both medical and veterinary importance. This tick is a vector to several human diseases, including *Rickettsia rickettsii* and *Rickettsia conorii* (Parker et al. 1933; Brumpt, 1932; Matsumoto et al., 2005; Parola et al., 2009), as well as diseases of veterinary importance such as *Babesia gibsoni, Babesia canis, Ehrlichia canis, and Hepatozoon canis* (Sen, 1933; Regendanz and Muniz, 1936; Groves et al., 1975; Nordgren and Craig, 1984; Demoner, 2013). The omnipresence of the tick's preferred host, the domestic dog (*Canis lupus familiaris*), likely contributes to its widespread distribution across many areas. The taxonomic history of *R. sanguineus* is complicated and has been the subject of much debate. The tick was originally named *Ixodes sanguineus* by Latreille (1806), based on a population in France. However, Latreille's description of the ticks was brief and vague. The taxonomic position of the tick was later transferred to the *Rhipicephalus* genus by Koch (1844), based on populations in Portugal, although this was accompanied yet again with little detail. The first detailed and comprehensive description of this species was given by Neumann (1897), based on morphological characteristics, with no mention of origin or specific behavior and geographic distribution (Barker and Walker, 2014). Despite its complex taxonomic history, *R. sanguineus* remains an important species to study due to its medical and veterinary significance. Understanding the distribution and behavior of this tick can help mitigate the spread of disease and improve public and animal health.

Studies over the past few decades have shown that populations identified as *Rhipicephalus sanguineus* based on classical morphological descriptions exhibit genetic and biological differences, including engorgement size, climate preference, and geographic isolation

(Cooley, 1946; Filippova, 1997; Pegram et al., 1987; Walker et al., 2000; Oliveira et al., 2005; Jones et al., 2017; Grant et al., 2023). Despite these differences, the taxonomic status of *R. sanguineus* remains uncertain. The original description of *R. sanguineus* lacked detail, and the specimens used by Latreille were not preserved or examined by subsequent authors who redescribed the tick. Furthermore, tick populations have been identified as *R. sanguineus* without following strict morphological, genetic, and biological criteria (Pegram et al., 1987; Farid, 1997; Nava et al., 2015). According to Camicas et al. (1998) and Walker et al. (2000), there are 17 different species within the *R. sanguineus* group.

Several genetic studies have differentiated *Rhipicephalus sanguineus* populations from various geographical localities (Szabo et al., 2005; Burlini et al., 2010; Moraes-Filho et al., 2011; Levin et al., 2012; Nava et al., 2012; Dantas-Torres, 2013). These studies concluded that there are two divergent lineages within *R. sanguineus* referred to as the temperate and tropical lineage. The temperate lineage included tick populations from Argentina, Uruguay, Chile, and Italy while the tropical lineage included ticks from Brazil, Paraguay, Colombia, South Africa, Mozambique, and two locations in Northern Argentina (Nava et al., 2012). A comprehensive morphological and genetic study was conducted on *R. sanguineus* from Europe, Asian, Africa, the Americas, and Oceania (Australia) as a continuation of the studies previously mentioned (Dantas-Torres, 2013). The results of this study concluded there is irrefutable evidence that these two lineages are different species under the name *R. sanguineus*. The results from Dantas-Torres (2013) were later supported by Nava et al. (2018), Tucker et al. (2020), Bakkes et al. (2020), Slapeta et al. (2021), and Walker et al. (2022). A recent study identified the tropical lineage as *Rhipicephalus linnaei* based on morphological characteristics described by Audouin, (1826) and genetic analysis using cox1, 12s rDNA, and 16S rDNA using *R. sanguineus* populations from Australia

(Slapeta and Chandra, 2021). A re-identification of the temperate lineage is yet to be confirmed. Both species will be referred to as the tropical and temperate lineage in this thesis due to its wide usage and the unresolved classification of the temperate lineage.

Morphologically, it was observed that both lineages differ in size, genital aperture, number of setae on the anal valves, mean size of chelicerae, size and distribution of chitinous plates, and festoon morphology (Oliveria, 2005; Szabó et al., 2005; Dantas-Torres, 2013). Szabó et al. (2005) conducted a study in South America to distinguish two dissimilar populations of *Rhipicephalus sanguineus* from Brazil, the tropical lineage, and Argentina, the temperate lineage. The engorged larvae, nymphs, and adults collected from Argentina where morphologically characterized as being heavier with a longer engorgement period by 50% in comparison to Brazil. The two populations were then cross mated, and a non-fertile female was produced. Other studies have indicated differences in climate preference between the two lineages (Parola et al., 2009; Dantas-Torres, 2015; Backus, 2021). The tropical lineage can be found in regions with an annual temperature of exceeding 20°C, while the temperate lineage is in regions with an annual temperature of 20°C or lower (Jones et al., 2017; Zemtsova, 2010; Backus, 2021). Backus (2021) explored how temperature influences host preference between tropical and temperate populations. The results indicated that the tropical lineage adults were 2.5 times more likely to migrate towards the human host at a higher temperature. The temperate lineage had a 66% reduction in preference for canine in high temperatures and a greater preference for humans at room temperature. The results of this study suggest that host choice and feeding behavior are influenced by the interaction between lineage and temperature.

The tropical and temperate lineage can be found across the United States (Centers for Disease Control and Prevention, 2019). Both lineages have been reported to be well delineated,

with no overlap (Eremeeva et al., 2011; Zemtsova et al., 2016; Jones et al., 2017; Hornok et al., 2017; Villarreal et al., 2018; Sanches-Montes, 2021). However, various studies in central eastern Arizona have found both the temperate and tropical groups living in sympatry (Jones et al., 2017; Rene-Martellet et al., 2017; Arizona Department of Health Services and Disease Control, 2021; Grant et al., 2023). Grant et al. (2023) investigated the geographic distribution of the tropical and temperate lineage in the United States based on collections from canines. The temperate lineage was present in Arkansas, California, Colorado, Idaho, Illinois, India, Kentucky, North Carolina, New Mexico, Ohio, Oklahoma, Oregon, Tennessee, Utah, Washington, and Wisconsin. The states reported to have the tropical lineage were Florida, Hawaii, Michigan, Minnesota, Wisconsin, Nevada, New York, Oklahoma, South Carolina. Texas and Arizona consisted of mixed infestations on canines; however, travel of pets may have led to a short-lived introduction of these two species within the same premises. The identification of the tropical lineage in the northern states Michigan and Minnesota with much cooler climate likely resulted from travel of canines with their owners. Specific cities or regions where the specimens were obtained in each state was not specified, therefore further research is needed to investigate if most of the tropical and temperate populations are geographically isolated between each state or by northern and southern regions within each state with limited sympatric populations. Similarly, Jones et al. (2017) identified the tropical and temperate lineages in different localities across the United States and yielded the same results from the states Arizona, California, Florida, Hawaii, Illinois, Oklahoma, and Texas. In Texas, the temperate lineage was found in Dallas, El Paso, Fort Worth, and San Angelo, while the tropical lineage was found in Edinburg. Further is research is needed to determine if the temperate lineage is geographically isolated to north Texas or if it is present in different localities in south Texas due to travel of companion canines.

The tropical and temperate species can be differentiated genetically, morphologically, biologically, and by its vectorial capacity therefore classifying them as two different species (Oliveira et al., 2005; Szabó et al., 2005; Dantas-Torres et al., 2013; Sanches et al., 2016). The aim of this work was to further investigate the lineage found in the Rio Grande Valley with a larger sample size and multiple collection sites. Past studies have indicated that both lineages are geographically isolated, however, new studies have indicated incidences where both lineages are within in the same area (Eremeeva et al., 2011; Zemtsova et al., 2016; Jones et al., 2017; Hornok et al., 2017; Villarreal et al., 2018; Sanches-Montes, 2021 Grant et al., 2023). This study will confirm if the temperate lineage is present in the Rio Grande Valley using 12S rRNA mitochondrial sequencing.

Methodology

Collections and Identification

Specimens were collected from three residential locations in the lower Rio Grande Valley. Sites 1 and 2 were both located in Mercedes, Texas ~7 miles apart while site 3 was in Edinburg, Texas ~42 miles from site 1 and 2 (Figure. 1B and 2B). Collections were conducted weekly in the afternoon from March 2021 to July 2021. The collection methods that were utilized were flagging and dragging, CO2 trap, and forceps. Flagging and dragging is a method relying on a tick's host-seeking behavior, known as questing, by dragging or sweeping a heavy cloth attached to a pole across tall grass or an infested area (Figure. 2B). The CO2 trap is a collection method that utilizes dry ice that emits CO2 to attract ticks. This trap can be described as a flat base with a Styrofoam box and double-sided tape around the box. The Styrofoam box will have holes near the bottom to release CO2. The double-sided tape around the Styrofoam

box will capture the ticks. This method can be further described by Yans et al. (2022). Forceps were utilized to collect off homes and canines belonging to the residential homeowners approved by IACUC protocol number: AUP-19-34 (Figure. 3B). The ticks were then stored in a -80 freezer and were identified by species and sex using a dichotomous key by Keirans and Litwak. (1989) based on tick populations present in the United States. A total of 250 adult ticks were collected and processed for DNA extractions. A statistical analysis using JMP software (SAS Institute Inc., 2019) was conducted using One-way ANOVA to compare the mean values of total weekly tick collections between each site, males and females by site, and engorgement status by site.

DNA Extractions

The *Rhipicephalus sanguineus* adult ticks collected from each site were then mechanically homogenized using a sterile scalpel. Blood was extracted from engorged female ticks using a 0.5 *m*l pipette before homogenizing. The whole specimen was utilized for DNA extraction. DNA extractions were then performed using the Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Valencia, California, USA). QIAGEN Supplementary Protocol: Purification of total DNA from ticks using the DNeasy® Blood and Tissue Kit for detection of Borrelia DNA steps one through ten were conducted with minor modifications. The QubitTM 2.0 Flurometer was used to identify quantity of DNA acquired from samples (Carlsbad, CA).

PCR Amplification and Sequencing

Gene amplification and sequencing were performed following protocols in Emborg and others. (2006). The 12S mitochondrial gene from each tick collected was amplified using the

GeneAmp TM PCR system (ABI Biosciences). The primers used for the amplification and sequencing of the 400 bp fragment were: forward, (5′- aaactaggattagataccctattattttag-3’) and reverse (5′-ctatgtaacgacttatcttaataaagagtg-3′) (Szabo et al., 2005). PCR conditions included 35 cycles of three temperatures consisting of two holds at 95 °C for 2 minutes. The initial duration first holds 95 °C for 1 minute, primer annealing at 50 °C for 1 minute, 72 °C for 1.5 minutes for primer extension, second hold, and 72 °C for 10 minutes to carry out a final extension. Negative controls were always run simultaneously. A 10 µl volume of the reaction mixture was examined by 1% agarose-gel electrophoresis followed by staining with SYBR green to validate the presence of amplified DNA. The amplified DNA was purified using Exo-Sap-It (Affymetrix, Santa Clara, California, USA) and sent to the University of Chicago Cancer Center DNA Sequencing and Genotyping Facility (Chicago, Illinois, USA) for sequencing on an ABI 3730 using BigDye Terminator (ABI, ThermoFisher, Waltham, Massachusetts, USA).

Sequence Analysis

Sequences were edited utilizing BioEdit 7.2 (Hall, 1999). Sequences were manually aligned and compared to one another utilizing 12S rRNA gene sequences of *R. sanguineus* previously deposited in GenBank. Once aligned, a subset of the 24 sequences was used to construct a phylogeny instead of all 250 sequences. A pairwise similarity matrix indicated between 99-100% similarity for all sequences. The outgroups for the phylogenetic analysis were KC243815 (*Rhipicephalus pusillus*) and KC243829 (*Rhipicephalus mushsamae*), the tropical lineage was represented by FJ536582 (Portugal) and KC243786 (South Africa), and the temperate lineage was represented by MH630345 (France) and KC243802 (Spain). jModeltest (2.1.10) (Darriba et al., 2012) was used to determine which model of molecular

evolution would be used for the subset of data and known sequences. AIC and BIC indicated that HKY85 + G + I would be the model that best fit. A maximum likelihood tree was constructed using PhyML 3.0 (Guindon et al., 2010) with a HKY85 + G + I model of molecular evolution. A standard bootstrap analysis of 1000 replicates were also selected. The resulting tree was visualized with iTOL v6.7.3 (Letunic & Bork, 2021).

Results

Ticks were collected over a period of 5 months during the summer of 2021, resulting in a total of 250 ticks confirmed to be *Rhipicephalus sanguineus* (see Table 1 and 2). No mixed populations of ticks were identified at any of the sites. The most effective collection method involved using forceps to collect ticks from residential homes and canines, while the CO2 trap, flagging, and dragging methods were unsuccessful. The CO2 trap did not work due to melting ice during field collections, while flagging and dragging did not work due to most of the ticks being on hosts or hiding in wall crevices. All sites had dogs present, and the average temperature in all localities ranged from 80-85 °F throughout the weekly collections (Weather underground, 2021). A statistical analysis using JMP software (SAS Institute Inc., 2019) was conducted using One-way ANOVA to compare the mean values of total weekly tick collections between each site, males and females by site, and engorgement status by site. The results were not statistically significant (P value > 0.05). Figure 9B presents a graph made using JMP software that shows the weekly tick collections from each site. Site 1 had the highest tick collection in week 13 (June), with the lowest collection period between week 3 and week 12. Site 2 had the highest tick collection in week 13, similar to site 1, and the lowest collection period between week 8 and week 11. Site 3 had its highest tick collection in week 3 (March) and its lowest collection period

between week 7 and week 11. The hindrance in tick collection was influenced by owners fumigating and administering tick preventative medication to their canines. In site 3, owners bathed their dogs weekly using tick preventative shampoo.

In this study, specimens were homogenized, and blood was extracted from engorged female ticks to ensure successful DNA extraction. The presence of DNA was confirmed via gel electrophoresis with SYBR green staining. Sequences from all 250 ticks were obtained and edited using BioEdit 7.2 sequencing software. Out of the 250 sequences obtained, 35 were not included in this study due to the presence of multiple undetected nucleotides "n" in the sequence analysis, which may have been caused by technical issues or errors during sequence analysis. Upon alignment, it was observed that there was no mitochondrial variation in the 12s rRNA gene among all ticks collected from each site (Figures 7B-9B).

The phylogenetic analysis included a total of 24 12S rRNA mitochondrial gene sequences from our collected samples (Figure 5B). The temperate lineage was not identified from the samples collected in the present study. The tropical and temperate lineages were separated into two divergent branches, indicating that they are two different species. The collected samples identified as the tropical lineage in all localities. Samples obtained from this study delineated into one cluster with KC243786 (South Africa) with a high bootstrap value (98%). Both tropical sequences had a high bootstrap value of 89% with FJ536582 (Portugal). Additionally, the sequence identity matrix analysis showed high levels of genetic similarity among the *Rhipicephalus sanguineus* collected from the three study sites, with sites 1 and 2 having an identity matrix of 1.0 (Figure 6B and 7B) and site 3 having a slightly lower identity matrix of 0.95 (Figure 8B), due to the presence of an undetected nucleotide "n" in the sequence analysis. This may have occurred due to technical issues or errors during sequence analysis that

can affect the results. The outgroup species KC243815 (*Rhipicephalus pusillus*) had a high bootstrap value of 0.80, indicating strong support for its placement on the phylogenetic tree. It was connected to a long branch with KC243829 (*Rhipicephalus mushsamae*) on one end and a short branch with a bootstrap value of 0.54 on the other end with the temperate lineages MH630345 (France) and KC243802 (Spain). The lower bootstrap value for this branch suggests lower confidence in its placement on the phylogenetic tree.

Discussion

This study successfully collected and analyzed *Rhipicephalus sanguineus* ticks in the Rio Grande Valley, discussing effective collection methods and genetic analysis of the 12S rRNA mitochondrial marker. The tick species was identified using a dichotomous key by Keirans and Litwak (1989). However, it should be noted that a more recent key by Walker et al. (2023) includes related species previously identified as *R. sanguineus,* such as *Rhipicephalus camicasi*, *Rhipicephalus guilhoni*, and *Rhipicephalus turanicus*, but does not cover the tropical and temperate lineages. One limitation of this study is the lack of morphological comparison between field-collected ticks and the morphological identification criteria established by Oliveira (2005) and Szabó et al. (2005) for the tropical and temperate lineages.

During field collections at residential homes, a majority of *R. sanguineus* adults and eggs were collected from the crevices in the walls (Figure 2B). These hidden areas are essential and provide the female and her eggs protection from predators such as spiders, birds, and wasps (Ramos et al., 2013; Dantas-Torres, 2010). The average temperature during field collections was consistently between 26.7-29.4°C across all collection weeks (Weather Underground, 2021).

Although temperature data were collected and analyzed alongside tick collections by month, no correlation was observed between temperature and tick yield. Therefore, it is unlikely that temperature had a significant effect on tick abundance during the study period. However, studies have reported that increased temperatures influence tick abundance and attachment (Pegram et al., 1987; Walker et al., 2000; Oliveira et al., 2005; Jones et al., 2017). Therefore, further research could be conducted with extended collection time and observation of the number of feeding ticks on a host in different temperatures.

The genetic results are consistent with previous studies that have shown low levels of genetic diversity among *Rhipicephalus sanguineus* populations in different geographic regions (Moraes-Filho et al., 2011; Dantas-Torres et al., 2013; Jones et al., 2017). The high sequence identity matrix observed in sites 1 and 2 (Figures 7B and 8B) may indicate that these sites have similar environmental conditions that favor the survival and reproduction of these tick populations, including optimal climate for the tropical species (>20 degrees °C), availability of hosts, and multiple areas of refuge for successful development. The slightly lower sequence identity matrix observed in site 3 may be attributed to the presence of an undetected nucleotide "n" during sequence analysis (Figure 9B). Despite this, the high degree of genetic similarity observed among the ticks from the three sites suggests that *R. sanguineus* collected from the sites are all the Tropical lineage. This may have implications for disease transmission and control as the tropical lineage has been reported to be a primary vector for *Rickettsia rickettsii* and *Ehrlichia canis* (Levin et al., 2012). Furthermore, the tropical lineage has shown pesticide resistance to permethrin and fipronil based on populations in Florida, the Caribbean, and North America (Tucker et al., 2021; Tian et al., 2023).

The results of this study provide evidence of the presence of the tropical lineage in South Texas, supporting the hypothesis that the climate in this region is suitable for this lineage. Conversely, the temperate lineage is commonly found in cooler areas (Zemtsova et al., 2016) and has been observed at a range of approximately 450 miles from our study sites (Jones et al., 2017). Further investigation is necessary to determine at what point along the range the temperate lineage becomes more prevalent. Jones et al. (2017) noted the presence of the tropical lineage in Edinburg, Texas with an annual average temperature above 23°C and the temperate lineage in El Paso, San Angelo, Fort Worth, and Dallas with an average annual temperature of 15-18°C (Weather underground, 2021). As previously indicated, the temperate lineage can survive in temperatures below 20°C (Zemtsova et al., 2016), and the tropical lineage can survive in above 20°C (Jones et al., 2017). Therefore, it is reasonable to predict that the tropical lineage will be present in areas south of Laredo and Corpus Christi, Texas, with annual temperature in mind. A safe hypothesis to make about the temperate lineage is that it is the only lineage present in the middle regions north of San Angelo and Tyler, Texas. This middle region has an annual temperature of 15-21°C (Weather underground, 2021). I hypothesize that the areas south of San Angelo, Waco, and Tyler, Texas, will be the tropical region with no mixed populations. Grant et al. (2023) reported mixed populations and two tropical tick populations in a northern region with cooler temperatures; however, further research is required to conclude that this applies to all populations. Future studies should focus on collecting more samples and expanding the study site to test this hypothesis.

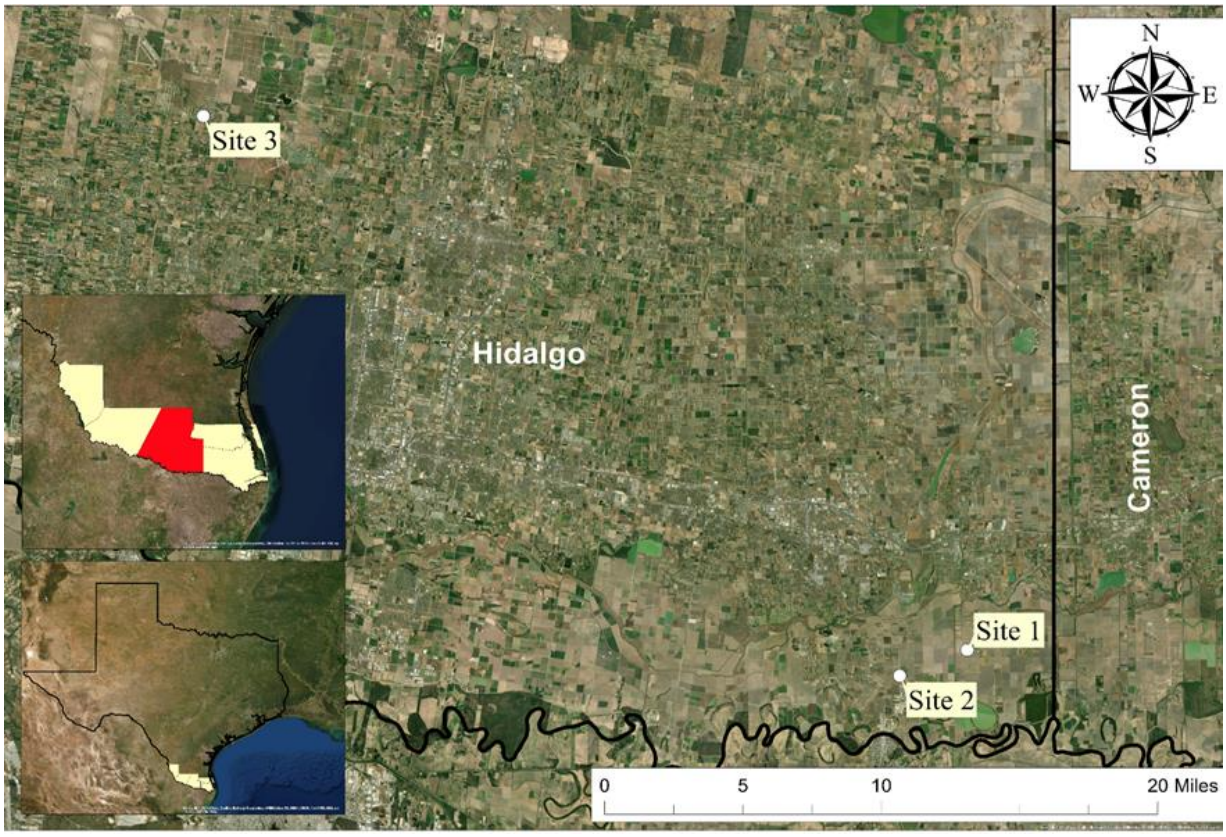


Figure 1B. This map depicts the locations of our field collections sites. The sites are in residential areas in South Texas. Sites 1-3 are labeled in the photo.

Figure 2B. This photo illustrates the dragging collection method utilized in this experiment.

Figure 3B. The photo exemplifies the flagging collection method used in this experiment.

Figure 4B. This photo portrays forceps being utilized for tick collections from residential homes.

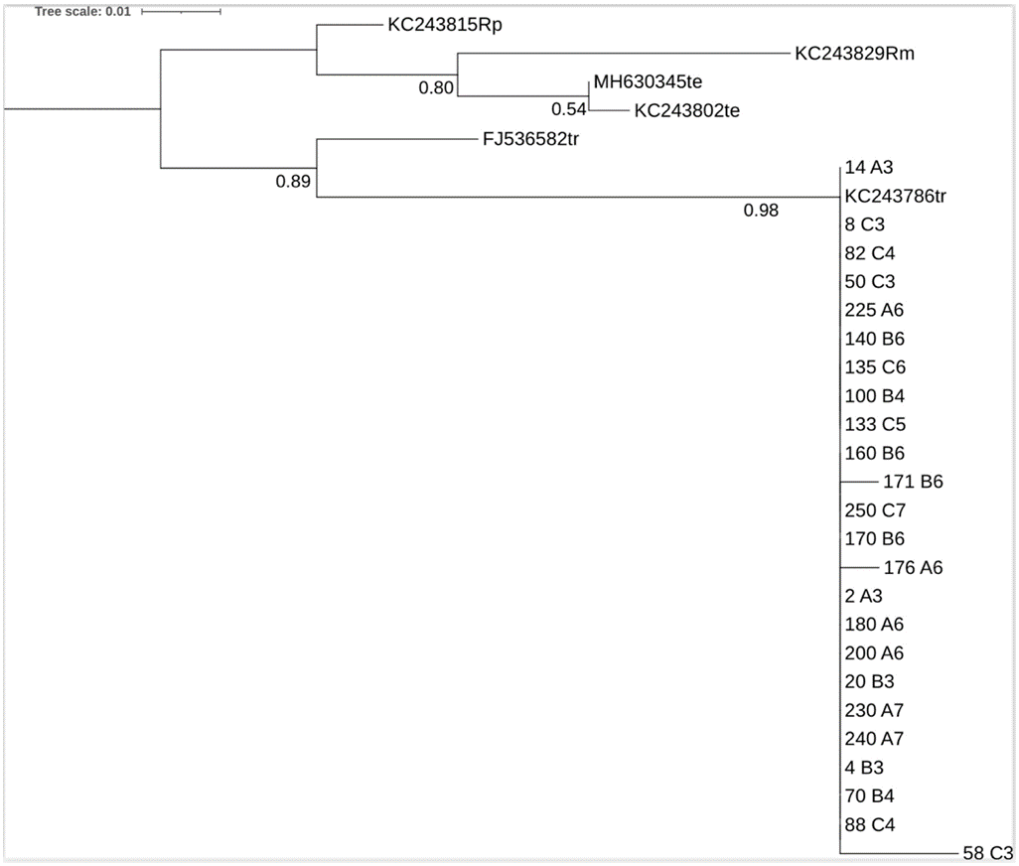
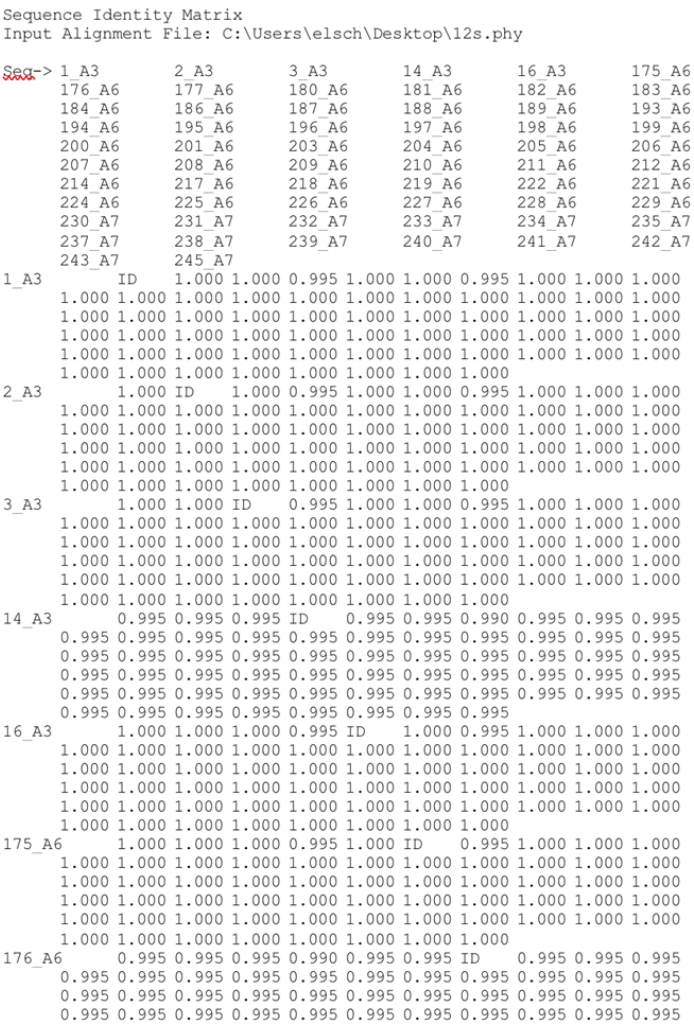


Figure 5B. Lineage of *Rhipicephalus sanguineus* from the Rio Grande Valley inferred from 12S rRNA sequences. Analysis was derived from ticks collected from this study and obtained from GenBank in North America and other regions. Ticks collected from this study representing with a number represented its collection number followed by a capitalized letter that represents site 1 (A), site 2 (B), and site 3 (C) and followed by another number indicating its collection month (ex. 100B4). Accession number and lineage is provided for some sequences (ex. KC243786tr and MH630345te) where tr signifies tropical and te stands for temperate. Bootstrap values are based on 1000 replicates with only bootstraps of >50% are indicated.



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Sequence Identity Matrix
Input Alignment File: C:\Users\elsch\Desktop\12s.phy

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      184_A6      186_A6      187_A6      188_A6      189_A6      193_A6
      194_A6      195_A6      196_A6      197_A6      198_A6      199_A6
      200_A6      201_A6      203_A6      204_A6      205_A6      206_A6
      207_A6      208_A6      209_A6      210_A6      211_A6      212_A6
      214_A6      217_A6      218_A6      219_A6      222_A6      221_A6
      224_A6      225_A6      226_A6      227_A6      228_A6      229_A6
      230_A7      231_A7      232_A7      233_A7      234_A7      235_A7
      237_A7      238_A7      239_A7      240_A7      241_A7      242_A7
      243_A7      245_A7
1_A3        ID    1.000 1.000 0.995 1.000 1.000 0.995 1.000 1.000 1.000
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2_A3        1.000 ID    1.000 0.995 1.000 1.000 0.995 1.000 1.000 1.000
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16_A3       1.000 1.000 1.000 0.995 ID    1.000 0.995 1.000 1.000 1.000
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175_A6      1.000 1.000 1.000 0.995 1.000 ID    0.995 1.000 1.000 1.000
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176_A6      0.995 0.995 0.995 0.990 0.995 0.995 ID    0.995 0.995 0.995
      0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995
      0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995
      0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995 0.995
```

Figure 6B. The photo presents the sequence identity matrix of site 1. Ticks collected from site 1 are represented with a number representing its collection number followed by a capitalized letter that represents site 1 (A) and followed by another number indicating its collection month (ex. 3_A3).

```
Sequence Identity Matrix
Input Alignment File: C:\Users\elsch\Desktop\12s.phy

Seq-> 4_B3        5_B3        6_B3        17_B3       18_B3       19_B3
      20_B3       20_B3       26_B3       70_B4       71_B4       72_B4
      73_B4       74_B4       75_B4       76_B4       77_B4       78_B4
      79_B4       80_B4       81_B4       89_B4       90_B4       91_B4
      92_B4       93_B4       94_B4       95_B4       96_B4       97_B4
      100_B4      101_B4      103_B4      104_B4      105_B4      106_B4
      108_B4      109_B4      111_B4      112_B4      113_B4      114_B4
      115_B4      116_B4      117_B4      118_B4      119_B4      120_B4
      121_B4      122_B4      123_B4      124_B4      125_B4      126_B4
      127_B4      129_B4      137_B6      138_B6      139_B6      140_B6
      141_B6      143_B6      144_B6      145_B6      146_B6      147_B6
      148_B6      149_B6      150_B6      151_B6      152_B6      156_B6
      157_B6      158_B6      153_B6      155_B6      159_B6      160_B6
      161_B6      162_B6      163_B6      164_B6      165_B6      167_B6
      168_B6      169_B6      170_B6      171_B6      172_B6      173_B6
      174_B6
4_B3        ID    1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      0.995 1.000 1.000 1.000
5_B3        1.000 ID    1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      0.995 1.000 1.000 1.000
6_B3        1.000 1.000 ID    1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      0.995 1.000 1.000 1.000
17_B3       1.000 1.000 1.000 ID    1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
      1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
```

Figure 7B. The sequence identity matrix of site 2 is illustrated in this photo. Ticks collected from site 2 are represented with a number representing its collection number followed by a capitalized letter that represents site 2 (B) and followed by another number indicating its collection month (ex. 4_B3).

Sequence Identity Matrix
Input Alignment File: C:\Users\elsch\Desktop\12s.phy

Seq->	7_C3	8_C3	28_C3	29_C3	30_C3	31_C3
	32_C3	38_C3	39_C3	40_C3	41_C3	42_C3
	43_C3	44_C3	49_C3	50_C3	51_C3	52_C3
	53_C3	54_C3	55_C3	58_C3	59_C3	60_C3
	61_C3	62_C3	63_C3	64_C3	65_C3	66_C3
	67_C3	69_C3	82_C4	83_C4	84_C4	85_C4
	88_C4	128_C5	130_C5	131_C5	132_C5	133_C5
	134_C6	135_C6	136_C6	246_C7	247_C7	248_C7
	249_C7	249_C7	250_C7			

7_C3	ID	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
8_C3	1.000	ID	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
28_C3	1.000	1.000	ID	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
29_C3	1.000	1.000	1.000	ID	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
30_C3	1.000	1.000	1.000	1.000	ID	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
31_C3	1.000	1.000	1.000	1.000	1.000	ID	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
32_C3	1.000	1.000	1.000	1.000	1.000	1.000	ID	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
38_C3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
39_C3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Figure 8B. The sequence identity matrix of site 3 is presented. The specimens collected from site 3 are represented with a number representing its collection number followed by a capitalized letter that represents site 3 (C) and followed by another number indicating its collection month (ex. 7_C3).

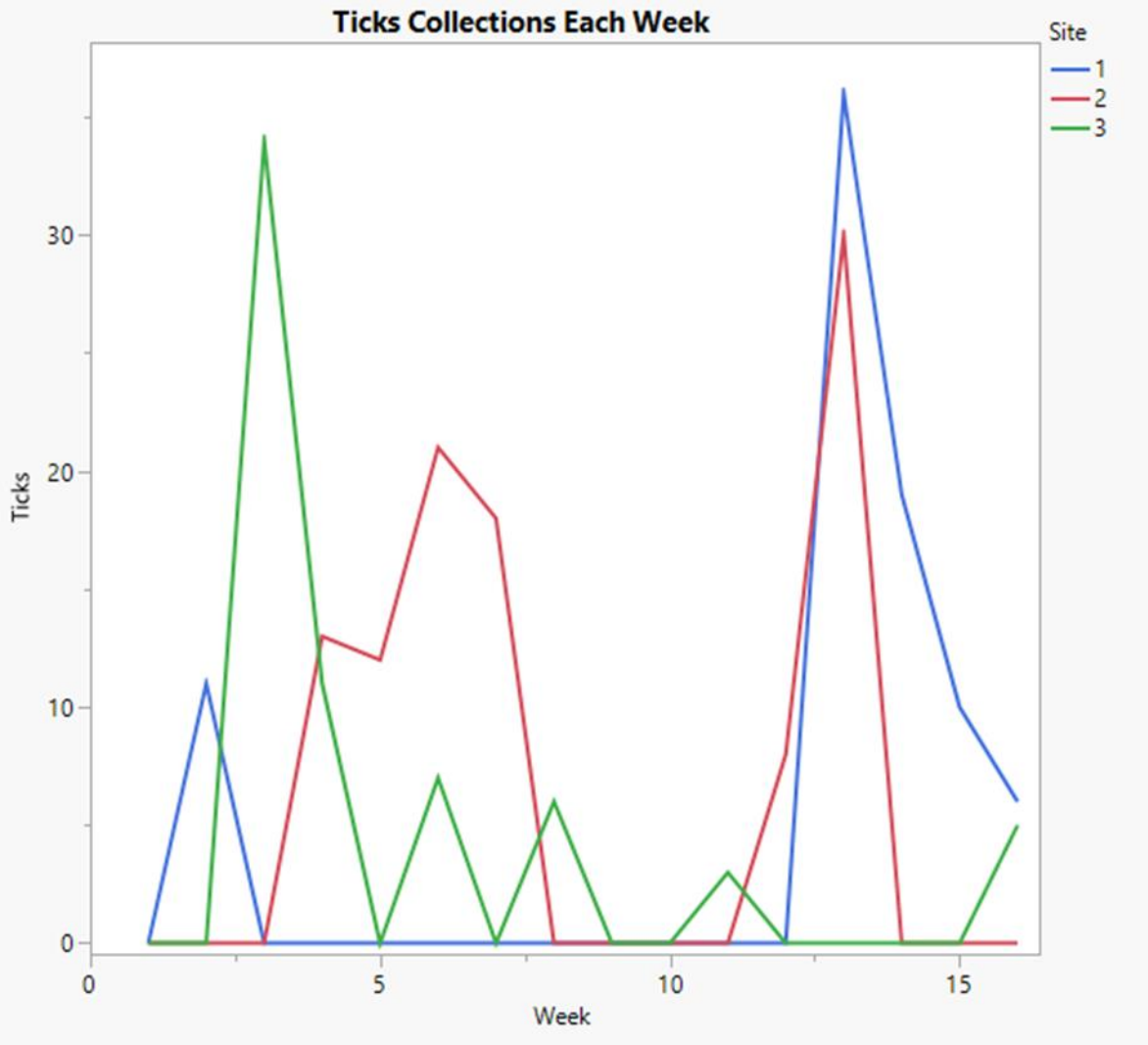

Figure 9B. The distribution of ticks collected per week among the three study sites. Two located in Mercedes TX and one in Edinburg TX is presented in this graph, created using JMP software.

Table 1. Total number of specimens collected from each site.

Site Number	Number of Ticks Collected
Site 1	82
Site 2	102
Site 3	66

Table 2. Total number of specimens collected monthly from each site.

Month	Site 1	Site 2	Site 3
March	11	13	45
April	0	51	7
May	0	0	5
June	55	38	3
July	16	0	6

CHAPTER III

SUMMARY AND CONCLUSION

The continued study of population genetics of *Rhipicephalus sanguineus* research is essential because they are the most common tick species across the globe and are primary vectors to diseases such as *Rickettsia rickettsii*, *Rickettsia conorii*, *Babesia gibsoni*, *Babesia canis, Ehrlichia canis*, and *Hepatozoon canis (*Parker et al., 1933; Sen, 1933; Brumpt, 1932; Regendanz and Muniz, 1936; Groves et al., 1975; Nordgren and Craig, 1984; Matsumoto et al., 2005; Parola et al., 2009; Demoner, 2013). A sequence analysis of 12s rRNA mitochondrial gene was used to identify the tropical or temperate lineages from three different populations in South Texas. The experimental hypothesis was that the study would confirm previous findings that there would be no temperate linage due to the warmer climate in the South Texas Region. The data presented in this study provides evidence that the tropical lineage is present in South Texas, as indicated by the high similarity of mitochondrial rRNA across all site localities.

From this research we can concludes that the tropical clade the only *Rhipicephalus* lineage present in the region. Previous research has reported the temperate clade can be found in four cities including Dallas, San Angelo, El Paso, and Forth Worth, located >450 miles north of the sites utilized in my study (Jones et al., 2017). The fact that the temperature lineage is found in Norther Texas suggest that there is a zone in Texas with overlap between the temperate and tropical lineage. Additional studies will need to be conducted to observe at which city or range between South Texas and North Texas will the temperate clade begin to be more present and is

the degree of overlap between the two clades. A comparative sequence analysis and morphological comparison of different populations can also be applied to observe if any variation is detected. Additional behavioral, physiological, and mating studies may help to confirm differences or similarities between these lineages that have been identified in other regions (). Ultimately, the spread and overlap of these two lineages may also help to identify disease potential as the vector competence varies between the lineages as well (Grant et al., 2023). Further studies are required to determine the degree of overlap, if the two lineages are unable to cross mate and produce viable offspring in Texas, and if the regions where the tick lineages are found is changing over time.

REFERENCES

Alvarez-Hernandez, G., Ernst, K., Acuna-Melendrez, N. H., Vargas-Ortega, A. P., & Candia-Plata, M. D. (2018). Medical knowledge related to Rocky Mountain spotted fever in Sonora, Mexico. Transactions of the Royal Society of Tropical Medicine and Hygiene, 112, 109-114.

Arizona Department of Health Services Epidemiology & Disease Control: Disease Data, Statistics, & Reports. [(accessed on 10 December 2021)];2021 Available online: https://azdhs.gov/preparedness/epidemiology-disease-control/index.php#data-stats-past-years

ArcMap desktop 10.7.1 system requirements https://desktop.arcgis.com/en/arcmap/latest/get-started/setup/arcgis-desktop-system-requirements.htm (2019)

Amyx, H. L., Huxsoll, D. L., Zeiler, D. C., & Hildebrandt, P. K. (1971). Therapeutic and prophylactic value of tetracycline in dogs infected with the agent of tropical canine pancytopenia. Journal of the American Veterinary Medical Association, 159, 1428-1432.

Ana Araya-Anchetta, J. D. Busch, G. A. Scoles, & D. M. Wagner. (2015). Thirty years of tick population genetics: A comprehensive review. Infection, Genetics and Evolution, 29, 164-179.

Aziz, M. U., Hussain, S., Song, B., Ghauri, H. N., Zeb, J., & Sparagano, O. A. (2022). Ehrlichiosis in dogs: A comprehensive review about the pathogen and its vectors with emphasis on South and East Asian countries. Veterinary Sciences, 10(1), 21.

Backus, L. H., López Pérez, A. M., & Foley, J. E. (2021). Effect of temperature on host preference in two lineages of the brown dog tick, Rhipicephalus sanguineus. The American Journal of Tropical Medicine and Hygiene, 104(6), 2305-2311. doi:10.4269/ajtmh.20-1376.

Barker, S. C. (1998). Distinguishing species and populations of rhipicephaline ticks with its 2 ribosomal RNA. Journal of Parasitology, 84, 887-892.

Beall, M. J., Alleman, A. R., Breitschwerdt, E. B., Cohn, L. A., Couto, C. G., Dryden, M. W., Guptill, L. C., Iazbik, C., Kania, S. A., Lathan, P., et al. (2012). Seroprevalence of Ehrlichia canis, Ehrlichia chaffeensis, and Ehrlichia ewingii in dogs in North America. Parasites & Vectors, 5, 29.

Beati L, Keirans JE. Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J Parasitol. 2001;87:32–48.

Brophy, M., Riehle, M. A., Mastrud, N., Ravenscraft, A., Adamson, J. E., & Walker, K. R. (2022). Genetic variation in Rhipicephalus sanguineus s.l. ticks across Arizona. International Journal of Environmental Research and Public Health, 19(7), 4223.

Brumpt, E. (1932). Longevite´ du virus de la fièvre boutonneuse (Rickettsia conorii, n. sp.) chez la tique Rhipicephalus sanguineus. Comptes Rendus de la Societe de Biologie, 110, 1197-1199.

Burlini L, Teixeira KR, Szabó MP, Famadas KM. Molecular dissimilarities of Rhipicephalus sanguineus (Acari: Ixodidae) in Brazil and its relation with samples throughout the world: is there a geographical pattern? Exp Appl Acarol. 2010;50:361–374. doi: 10.1007/s10493-009-9321-8.

Camicas JL, Hervy JP, Adam F, Morel PC. Les tiques du monde. Nomenclature, stades décrits, hôtes, répartition (Acarida, Ixodida) Paris: Éditions de l'Orstom; 1998.

Centers for Disease Control and Prevention Tickborne Disease Surveillance Data Summary. [(accessed on 11 December 2019)];2019 Available online: https://www.cdc.gov/ticks/data-summary/index.html

Centers for Disease Control and Prevention. (n.d.). Rhipicephalus sanguineus: Brown dog tick. Retrieved from https://www.cdc.gov/ticks/pdfs/Rhipicephalus-sanguineus-LifeCycle.pdf

Cooley RA. The genera Boophilus, Rhipicephalus and Haemaphysalis (Ixodidae) of the New World . Washington, D.C: National Institute of Health; 1946.

Dantas-Torres, F. (2007). Rocky Mountain spotted fever. Lancet Infectious Diseases, 7, 724-732.

Dantas-Torres, F. (2008). The brown dog tick, Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. Veterinary Parasitology, 152, 173-185.

Dantas-Torres, F. (2010). Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. Parasites & Vectors, 3, 26.

Dantas-Torres, F. (2015). Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. International Journal of Parasitology: Parasites and Wildlife, 4, 452-461.

Dantas-Torres, F., Annoscia, G., & Otranto, D. (2013). Morphological and genetic diversity of Rhipicephalus sanguineus sensu lato from the New and Old Worlds. Parasites & Vectors, 6, 213.

Dantas-Torres, F., Figueredo, L. A., & Brandão-Filho, S. P. (2006). Rhipicephalus sanguineus (Acari: Ixodidae), the brown dog tick, parasitizing humans in Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 39, 64-67.

Dantas-Torres, F., Latrofa, M. S., Annoscia, G., & Otranto, D. (2015). Further thoughts on the taxonomy and vector role of Rhipicephalus sanguineus group ticks. Veterinary Parasitology, 208, 9-13.

Dantas-Torres, F., Latrofa, M. S., Ramos, R. A. N., & Otranto, D. (2018). Biological compatibility between two temperate lineages of brown dog ticks, Rhipicephalus sanguineus (sensu lato). Parasites & Vectors, 11, 398.

Dantas-Torres, F., Latrofa, M. S., Weigl, S., Tarallo, V. D., Lia, R. P., & Otranto, D. (2012). Hepatozoon canis infection in ticks during spring and summer in Italy. Parasitology Research, 110, 695-698.

Dantas-Torres, F., Melo, M. F., Figueredo, L. A., & Brandão-Filho, S. P. (2009). Ectoparasite infestation on rural dogs in the municipality of São Vicente Férrer, Pernambuco, Northeastern Brazil. Revista Brasileira de Parasitologia Veterinária, 18, 75-77.

De la Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A. G., Estrada-Peña, A., Johnson, N., Kocan, K. M., Mansfield, K. L., Nijhof, A. M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllón, N., Vancova, M., Golovchenko, M., Grubhoffer, L., Caracappa, S., Rego, R. (2017). Tick-Pathogen Interactions and Vector Competence: Identification of Molecular Drivers for Tick-Borne Diseases. Frontiers in cellular and infection microbiology, 7, 114.

Demma, L. J., Holman, R. C., McQuiston, J. H., et al. (2005). Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001-2002. Am J Trop Med Hyg, 73, 400-409.

Demma LJ, Traeger MS, Nicholson WL, Paddock CD, Blau DM, Eremeeva ME, Dasch GA, Levin ML, Singleton JJ, Zaki SR, Cheek JE, Swerdlow DL, McQuiston JH. (2005). Rocky Mountain spotted fever from an unexpected tick vector in Arizona. New England Journal of Medicine, 353, 587-594.

Demoner, L. C., Rubini, A. S., Paduan, K. S., Metzger, B., de Paula Antunes, J. M., Martins, T. F., Mathias, M. I., O'Dwyer, L. H. (2013). Investigation of tick vectors of Hepatozoon canis in Brazil. Ticks Tick Borne Dis, 4, 542–546.

DNeasy Blood & Tissue Kits. (n.d.). Retrieved January 30, 2019, from https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/dneasy-blood-and-tissue-kit/#orderinginformation

Eremeeva, M. E., Zambrano, M. L., Anaya, L., Beati, L., Karpathy, S. E., Santos-Silva, M. M., Salceda, B., MacBeth, D., Olguin, H., Dasch, G. A., Aranda, C. A. (2011). Rickettsia rickettsii in Rhipicephalus ticks, Mexicali, Mexico. J. Med. Entomol., 48, 418–421.

Emborg, J., Dalgaard, P., & Ahrens, P. (2006). Morganella psychrotolerans sp. nov., a histamine-producing bacterium isolated from various seafoods. International Journal of Systematic and Evolutionary Microbiology, 56(10), 2473-2479.

Farid HA. Morphological keys for the separation of the Rhipicephalus sanguineus group of ticks (Acarina: Ixodidae) in Egypt. J Egypt Soc Parasitol. 1996;26:453–460

Feldman-Muhsam, B. (1952). On the identity of Rhipicephalus sanguineus Lat. Bull. Res. Council Israel, 2, 187–194.

Feldman-Muhsam B, Borut S. (1971). Population in ixodid ticks. J Parasitol., 57, 630-634. doi:

Filippova NA. Ixodid ticks of subfamily Amblyomminae. Fauna of Russia and neighboring countries. St. Petersburg: Nauka Publishing House; 1997 10.2307/3277930.

Giannelli, A., Ramos, R. A., Di Paola, G., Dantas-Torres, F., Baneth, G., Otranto, D. (2013). Transstadial transmission of Hepatozoon canis from larvae to nymphs of Rhipicephalus sanguineus. Vet. Parasitol., 196, 1–5.

Gray, J. S., Dantas-Torres, F., Estrada-Peña, A., Levin, M. (2013). Systematics and ecology of the brown dog tick, Rhipicephalus sanguineus. Ticks Tick-Borne Dis, 4

Gray JS, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. (2009). Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis.

Grant, A. N., Lineberry, M. W., Sundstrom, K. D., Allen, K. E., & Little, S. E. (2023). Geographic Distribution and Seasonality of Brown Dog Tick Lineages in the United States. Journal of medical entomology, 60(1), 102–111.

Hall, T. (1999). BioEdit version 7.2. 0. Distributed by the author, website: www. mbio. ncsu. edu/BioEdit/bioedit. html.

Hadani, A., & Rechav, Y. (1969). Tick-host relationships. 1. The existence of a circadian rhythm of "drop-off" of engorged ticks from their hosts. Acta Tropica, 26, 173-179.

Hadi, U. K., & Adventini, M. (2015). Fecundity, oviposition and egg incubation period of female Rhipicephalus sanguineus Latreille (Acari: Ixodidae) ticks in Indonesia. Journal of Veterinary Medicine Research, 2(5), 1036.

Hornok S., Sándor A.D., Tomanović S., Beck R., D'Amico G., Kontschán J., Takács N., Görföl T., Bendjeddou M.L., Földvári G., et al. East and west separation of Rhipicephalus sanguineus mitochondrial lineages in the Mediterranean Basin. Parasites Vectors. 2017;10:39. doi: 10.1186/s13071-017-1985-z.

Inokuma H., Beppu T., Okuda M., Shimada Y., Sakata Y. Epidemiological survey of Anaplasma platys and Ehrlichia canis using ticks collected from dogs in Japan. Vet. Parasitol. 2003;115:343–348. doi: 10.1016/S0304-4017(03)00238-3.

Ioffe-Uspensky, I., Mumcuoglu, K. Y., Uspensky, I., & Galun, R. (1997). Rhipicephalus sanguineus and R. turanicus (Acari: Ixodidae): closely related species with different biological characteristics. Journal of Medical Entomology, 34, 74-81.

Jones, E. O., Gruntmeir, J. M., Hamer, S. A., & Little, S. E. (2017). Temperate and tropical lineages of brown dog ticks in North America. Veterinary Parasitology, Regional Studies and Reports, 7, 58-61.

Jongejan, F., & Uilenberg, G. (2004). The global importance of ticks. Parasitology, 129(Suppl 1), S3-S14.

Keirans, J.E. and Litwak, T.R. (1989) Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), East of the Mississippi River. *Journal of Medical Entomology* 26: 435-448.

Koch CL. Systematische Uebersicht über die Ordnung der Zecken. Arch Naturgesch. 1844; 10:217–239.

Koch, H. G. (1982). Seasonal incidence and attachment sites of ticks (Acari: Ixodidae) on domestic dogs in southeastern Oklahoma and northwestern Arkansas, USA. Journal of medical entomology, 19(3), 293–298.

Koch, H. G. (1982). Oviposition of the brown dog tick (Acari: Ixodidae) in the laboratory. Annals of the Entomological Society of America, 75(5), 583-586.

Koch, H. G., & Tuck, M. D. (1986). Molting and survival of the brown dog tick (Acari: Ixodidae) under different temperatures and humidities. Annals of the Entomological Society of America, 79(1), 11-14.

Komnenou, A. A., Mylonakis, M. E., Kouti, V., Tendoma, L., Leontides, L., Skountzou, E., ... & Koutinas, A. F. (2007). Ocular manifestations of natural canine monocytic ehrlichiosis (Ehrlichia canis): A retrospective study of 90 cases. Veterinary ophthalmology, 10(3), 137-142.

Kordick, S. K., Breitschwerdt, E. B., Hegarty, B. C., Southwick, K. L., Colitz, C. M., Hancock, S. I., ... & MacCormack, J. N. (1999). Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. Journal of clinical microbiology, 37(8), 2631-2638.

Labruna, M. B., Gerardi, M., Krawczak, F. S., & Moraes-Filho, J. (2017). Comparative biology of the tropical and temperate species of Rhipicephalus sanguineus sensu lato (Acari: Ixodidae) under different laboratory conditions. Ticks and tick-borne diseases, 8(1), 146–156.

Latrofa, M. S., Dantas-Torres, F., Giannelli, A., & Otranto, D. (2014). Molecular detection of tick-borne pathogens in Rhipicephalus sanguineus group ticks. Ticks and tick-borne diseases, 5(6), 943–946.

Latreille, P. A. (1806). Genera crustaceorum et insectorum secundum ordinem naturalem in familia disposita, iconibus exemplisque plurimis explicata. Paris et Argentorati, 1, 302.

Levin, M. L., Studer, E., Killmaster, L., Zemtsova, G., & Mumcuoglu, K. Y. (2012). Crossbreeding between different geographical populations of the brown dog tick, Rhipicephalus sanguineus (Acari: Ixodidae). Experimental & Applied Acarology, 58(1), 51–68.

Little, S. E., Hostetler, J., & Kocan, K. M. (2007). Movement of Rhipicephalus sanguineus adults between co-housed dogs during active feeding. Veterinary Parasitology, 150(1-2), 139–145.

Lori, A., Lanfranchi, P., & Manilla, G. (1996). Contribution to the knowledge of Ixodidae ticks of wild mammals of Somalia. Parassitologia, 38, 571–573.

Louly, C. C., Soares, S. F., da Nóbrega Silveira, D., Guimarães, M. S., & Borges, L. M. (2010). Differences in the behavior of Rhipicephalus sanguineus tested against resistant and susceptible dogs. Experimental & applied acarology, 51(4), 353–362.

Louly, C. C. B., Fonseca, I. N., Oliveira, V. F., Linhares, G. F. C., Menezes, L. B., & Borges, L. M. F. (2007). Seasonal dynamics of *Rhipicephalus sanguineus* (Acari: Ixodidae) in dogs from a police unit in Goiania, Goias, Brazil. Ciência Rural, 37, 464-469.

Mans, B. J., & Neitz, A. W. (2004). Adaptation of ticks to a blood-feeding environment: evolution from a functional perspective. Insect Biochemistry and Molecular Biology, 34, 1-17.

Matias, P. J., Szabó, M. P., Mangold, A. J., João, C. F., Bechara, G. H., & Guglielmone, A. A. (2005). Biological and DNA evidence of two dissimilar populations of the Rhipicephalus sanguineus tick group (Acari: Ixodidae) in South America. Veterinary Parasitology, 130(1-2), 131-140.

Matsumoto, K., Brouqui, P., Raoult, D., & Parola, P. (2005). Experimental infection models of ticks of the Rhipicephalus sanguineus group with Rickettsia conorii. Vector-Borne and Zoonotic Diseases, 5, 363-372.

Mentz, M. B., Trombka, M., Silva, G. L., & Silva, C. E. (2016). Rhipicephalus sanguineus (ACARI: IXODIDAE) BITING A HUMAN BEING IN PORTO ALEGRE CITY, RIO GRANDE DO SUL, BRAZIL. Revista do Instituto de Medicina Tropical de Sao Paulo, 58, 35.

Moraes-Filho, J., Marcili, A., Nieri-Bastos, F. A., Richtzenhain, L. J., & Labruna, M. B. (2011). Genetic analysis of ticks belonging to the *Rhipicephalus sanguineus* group in Latin America. Acta Tropica, 117, 51-55.

Moraes-Filho, J., Krawczak, F. D. S., Costa, F. B., Soares, J. F., & Labruna, M. B. (2015). Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. PloS one, 10(9), e0139386.

National Weather Services. (2021). Brief Weather and Climate Review for the Rio Grande Valley: A Year of Extremes, New Records, and Over 1 Billion in Losses-Again. https://www.weather.gov/media/bro/wxevents/2021/pdf/RGV2021WeatherClimateSummary.pdf

Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M. B., Szabó, M. P., Venzal, J. M., Mastropaolo, M., Mangold, A. J., & Guglielmone, A. A. (2015). The taxonomic status of Rhipicephalus sanguineus (Latreille, 1806). Veterinary Parasitology, 208(1-2), 2-8.

Nava, S., Mastropaolo, M., Venzal, J. M., Mangold, A. J., & Guglielmone, A. A. (2012). Mitochondrial DNA analysis of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) in the Southern Cone of South America. Veterinary Parasitology, 190, 547–555.

Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M. B., Szabó, M. P., Venzal, J. M., Mastropaolo, M., Mangold, A. J., & Guglielmone, A. A. (2015). The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). Veterinary Parasitology, 208(1-2), 2–8. doi: 10.1016/j.vetpar.2014.12.017

Neumann, L. G. (1911). Ixodidae. Das Tierreich, 31. Berlin: R. Friedländer & Sohn.

Ndip, L. M., Ndip, R. N., Esemu, S. N., Walker, D. H., & McBride, J. W. (2010). Predominance of Ehrlichia chaffeensis in Rhipicephalus sanguineus ticks from kennel-confined dogs in Limbe, Cameroon. Experimental and Applied Acarology, 50(2), 163-168.

Ndip, L. M., Ndip, R. N., Ndive, V. E., Awuh, J. A., Walker, D. H., & McBride, J. W. (2007). Ehrlichia species in Rhipicephalus sanguineus ticks in Cameroon. Vector-borne and zoonotic diseases (Larchmont, N.Y.), 7(2), 221–227. doi: 10.1089/vbz.2006.0611.

Nordgren, R.M., Craig, T.M. (1984). Experimental transmission of the Texas strain of Hepatozoon canis. Vet. Parasitol. 16, 207–214.

de Oliveira, P. R., Bechara, G. H., Denardi, S. E., Saito, K. C., Nunes, E. T., Szabó, M. P., & Mathias, M. I. (2005). Comparison of the external morphology of Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodidae) ticks from Brazil and Argentina. Veterinary parasitology, 129(1-2), 139–147.

Oliver, J. H. (1989). Biology and systematics of ticks (Acari: Ixodida). Annu Rev Ecol Syst. 20: 397-430.

Parker, R. R., Philip, C. B., & Jellison, W. L. (1933). Rocky Mountain spotted fever: potentialities of tick transmission in relation to geographical occurrence in the United States. American Journal of Tropical Medicine and Hygiene, 13, 341–379.

Parola, P., Raoult, D. (2001). Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clinical Infectious Diseases, 32, 897–928.

Parola, P., Socolovschi, C., Jeanjean, L., Bitam, I., Fournier, P. E., Sotto, A., Labauge, P., Raoult, D. (2008). Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Neglected Tropical Diseases, 2, e338.

Parola, P., Socolovschi, C., Raoult, D. (2009). Deciphering the relationships between Rickettsia conorii conorii and Rhipicephalus sanguineus in the ecology and epidemiology of Mediterranean spotted fever. Rickettsiology and Rickettsial Diseases 1166, 49-54.

Paz, G. F., Labruna, M. B., & Leite, R. C. (2008). Ritmo de queda de Rhipicephalus sanguineus (Acari: Ixodidae) de cães artificialmente infestados. Revista Brasileira de Parasitologia Veterinária, 17, 139-144.

Pegram, R. G., Clifford, C. M., Walker, J. B., & Keirans, J. E. (1987a). Clarification of the Rhipicephalus sanguineus group (Acari, Ixodoidea, Ixodidae). I. R. sulcatus Neumann, 1908 and R. turanicus Pomerantsev, 1936. Systematic Parasitology, 10, 3–26.

Pegram, R. G., Keirans, J. E., Clifford, C. M., & Walker, J. B. (1987b). Clarification of the Rhipicephalus sanguineus group (Acari, Ixodoidea, Ixodidae). II. R. sanguineus (Latreille, 1806) and related species. Systematic Parasitology, 10, 27–44.

Qubit® 2.0 Fluorometer User Manual. Calsbad: Invitrogen™, 2010

Ramos, R. A., Giannelli, A., Dantas-Torres, F., & Otranto, D. (2013). Effect of egg clustering on the fitness *of Rhipicephalus sanguineus* larvae. Parasitology research, 112(4), 1795–1797.

Rechav, Y., & Nuttall, P. A. (2000). The effect of male ticks on the feeding performance of immature stages of Rhipicephalus sanguineus and Amblyomma americanum (Acari: Ixodidae). Experimental & Applied Acarology, 24, 569-578.

Regendanz, P., & Muniz, J. (1936). O Rhipicephalus sanguineus como transmissor da piroplasmose canina no Brasil. Memórias do Instituto Oswaldo Cruz, 31, 81-84.

René-Martellet, M., Minard, G., Massot, R., Van, V. T., Moro, C. V., Chabanne, L., & Mavingui, P. (2017). Bacterial microbiota associated with *Rhipicephalus sanguineus* (s.l.) ticks from France, Senegal and Arizona. Parasites & vectors, 10(1), 416.

Sanches, G. S., Evora, P. M., Mangold, A. J., Jittapalapong, S., Rodriguez-Mallon, A., Guzman, P. E., Bechara, G. H., & Carmarga-Mathias, M. I. (2016). Molecular, biological, and morphometric comparisons between different geographic populations of Rhipicephalus sanguineus sensu lato. Veterinary Parasitology, 215, 78-87.

Sanchez-Montes, S. (2021). *Rhipicephalus sanguineus* Complex in the Americas: Systematic, Genetic Diversity, and Geographic Insights. Pathogens, 10, 1118.

Sanogo, Y.O., Davoust, B., Inokuma, H., Camicas, J.-L., Parola, P., & Brouqui, P. (2003). First evidence of Anaplasma platys in *Rhipicephalus sanguineus* (Acari: Ixodida) collected from dogs in Africa. Onderstepoort Journal of Veterinary Research, 70, 205-212.

SAS Institute Inc. (2019). JMP® Version 13.0.0. Cary, NC: SAS Institute Inc.

Schaefer C, Allen J, Yao T, Owen H, Lisowski S, VandenBrooks J. (2019). The phylogenetics of *Rhipicephalus sanguineus* and its role as a vector of Rocky Mountain spotted fever. FASEB J 33: lb296.

Sen, S. K. (1933). The vector of canine piroplasmosis due to Piroplasma gibsoni. Indian Journal of Veterinary Science & Animal Husbandry, 3, 356-363.

Šlapeta, J., Chandra, S., & Halliday, B. (2021). The "tropical lineage" of the brown dog tick Rhipicephalus sanguineus sensu lato identified as Rhipicephalus linnaei. International Journal for Parasitology, 51(6), 431–436.

Šlapeta, J., Halliday, B., Chandra, S., Alanazi, A. D., & Abdel-Shafy, S. (2022). Rhipicephalus linnaei (Audouin, 1826) recognised as the "tropical lineage" of the brown dog tick Rhipicephalus sanguineus sensu lato: Neotype designation, redescription, and establishment of morphological and molecular reference. Ticks and Tick-borne Diseases, 13(6), 102024. Advance online publication.

Sykes, J. E. (2014). Ehrlichiosis. In J. E. Sykes (Ed.), Canine and Feline Infectious Diseases (pp. 278-289). W.B. Saunders. ISBN 9781437707953.

Snowden, J., & Simonsen, K.A. (2022). Rickettsia Rickettsiae. In StatPearls. StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK430881/

Straily, A., Drexler, N., Cruz-Loustaunau, D., Paddock, C. D., & Alvarez-Hernandez, G. (2016). Notes from the Field: Community-Based Prevention of Rocky Mountain Spotted Fever - Sonora, Mexico, 2016. MMWR. Morbidity and mortality weekly report, 65(46), 1302–1303.

Stiles, J. (2000). Canine Rickettsial Infections. Veterinary Clinics of North America - Small Animal Practice, 30, 1135-1149.

Stoffel, R. T., McClure, J. C., Butcher, M. M., Johnson, G. C., Roland, W., Cheng, C., Sirigireddy, K. R., Ganta, R., Boughan, K., Ewing, S. A., & Stich, R. W. (2014). Experimental infection of Rhipicephalus sanguineus with Ehrlichia chaffeensis. Veterinary microbiology, 172(1-2), 334–338.

Szabo, M.P., Bechara, G.H. (1999). Sequential histopathology at the Rhipicephalus sanguineus tick feeding site on dogs and guinea pigs. Experimental and Applied Acarology, 23, 915-928. doi: 10.1023/A:1006347200373.

Szabo, M.P., Mangold, A.J., Joao, C.F., Bechara, G.H., Guglielmone, A.A. (2005). Biological and DNA evidence of two dissimilar populations of the Rhipicephalus sanguineus tick group (Acari: Ixodidae) in South America. Veterinary Parasitology, 130, 131-140.

Thermo Fisher Scientific. (2016). ABI. Retrieved April 11, 2023, from https://www.thermofisher.com/us/en/home/brands/applied-biosystems.html

Thermo Fisher Scientific. (2016). Affymetrix. Retrieved April 11, 2023, from https://www.thermofisher.com/us/en/home/brands/affymetrix.html

Tian, Y., Taylor, C. E., Lord, C. C., & Kaufman, P. E. (2023). Evidence of Permethrin Resistance and Fipronil Tolerance in *Rhipicephalus sanguineuss.*l. (Acari: Ixodidae) Populations From Florida and California. Journal of Medical Entomology, 60(2), 412–416.

Tian, Y., Lord, C., & Kaufman, P. E. (2020). Brown Dog Tick Rhipicephalus Sanguineus Latreille (Arachnida: Acari: Ixodidae): EENY-221/IN378, Rev. 2/2020. EDIS, 2020(2), 6.

Villarreal, Z., Stephenson, N., & Foley, J. (2018). Possible northward introgression of a tropical lineage of Rhipicephalus sanguineus ticks at a site of emerging Rocky Mountain spotted fever. The Journal of Parasitology, 104, 240-245.

Walker, D. H. (2005). Ehrlichia under our noses and no one notices. Archives of Virology. Supplementum, 19, 147-156.

Walker, J. B., Keirans, J. E., & Horak, I. G. (2000). Genus Rhipicephalus (Acari, Ixodidae): A guide to the brown ticks of the world. Cambridge University Press.

Weather Underground. (2021). Monthly Averages for Edinburgh, TX. Weather Underground. https://www.wunderground.com/history/monthly/us/tx/edinburg/KEBG/date/2021-1

Wikswow, M. E., Hu, R. J., Metzger, M. E., & Eremeeva, M. E. (2007). Detection of Rickettsia rickettsii and Bartonella henselae in Rhipicephalus sanguineus ticks from California. Journal of Medical Entomology, 44, 158-162.

Yans, M. W., Branca, A. S., Hahn, N. G., Crawley, S. E., Figurskey, A. C., Hobson, K. R., Banfield, M. G., & Borden, J. H. (2022). Development of a Simple Trap That Captures Ticks (Acari) on Their Dorsal Surface. Journal of medical entomology, 59(3), 969–975.

Zahler, M., Filippova, N. A., Morel, P. C., Gothe, R., & Rinder, H. (1997). Relationships between species of the Rhipicephalus sanguineus group: A molecular approach. The Journal of Parasitology, 83, 302-306.

Zemtsova, G., Killmaster, L. F., Mumcuoglu, K. Y., & Levin, M. L. (2010). Co-feeding as a route for transmission of Rickettsia conorii israelensis between Rhipicephalus sanguineus ticks. Experimental and Applied Acarology, 52, 383-39.

BIOGRAPHICAL SKETCH

Bianca Liana Guerra attended the University of Texas Rio Grande Valley where she reviewed her Bachelor of Science degree in Biology in May 2020. During her undergraduate years, she enjoyed volunteering in the Vector Borne Diseases Laboratory where she had the privilege to assist in mosquito pesticide resistance research for the Texas Department of State Health Services (DSHS) and tick surveillance with the Centers for Disease Control and Prevention. After graduation, she wanted to continue her education and advance her skills in research. Bianca attended the University of Texas Rio Grande Valley where she earned her Master of Science in Biology in May 2023. Her thesis focused on population genetics of *Rhipicephalus sanguineus* in South Texas. While completing her master's degree, Bianca continued to assist in mosquito pesticide resistance research for the Texas Department of State Health Services (DSHS) and tick surveillance with the Centers for Disease Control and Prevention for the first year of graduate school. Bianca then interned at the UTRGV Biomedical Research Center where she conducted research on cervical cancer and HPV. On her free time, she enjoys CrossFit and playing with her golden retriever Lola. Bianca plans on continuing her education in biology and hopes to receive a PhD in molecular biology.

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