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Baseline biodiversity assessment of South Texas small mammals and host-associated hard ticks with no detection of selected tick-borne pathogens

Aleyda P. Galán[1,2], Sarah A. Hamer[3], Hunter A. Folmar[1,4], Tyler A. Campbell[5], and Jessica E. Light[1,*]

[1]*Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843*
[2]*ConnectGen, Houston, TX 77002*
[3]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843*
[4]*Florida Fish and Wildlife Conservation Commission, Punta Gorda, FL 33955*
[5]*East Foundation, San Antonio, TX 78216*

Abstract.—Baseline biodiversity surveys are necessary to assess organismal diversity across spatial and temporal scales. These surveys can be particularly useful for monitoring changes in organismal diversity and pathogen spread in response to climate change. Arthropod vectors such as ticks are susceptible to geographic range shifts with a warming climate, potentially resulting in the expansion of risk areas for vector-borne disease. Biodiversity data are deficient from South Texas, which is particularly concerning given the abundance of wildlife and livestock that may be important in perpetuating tick and pathogen populations. We performed a baseline biodiversity assessment of small mammals, ticks, and tick-borne pathogens in South Texas using a combination of fieldwork, collections-based research, and molecular approaches. We recorded 19 species of small mammals and 3 species of ticks, and we detected no tick-borne pathogens belonging to the genera *Borrelia* or *Rickettsia* in the ticks or mammals. Given the continued emergence of tick-borne disease, we recommend collaborations with natural history collections and private landowners interested in land stewardship so researchers can develop a better understanding of changing small mammal, tick, and pathogen diversity with implications for human and veterinary health in this region of Texas.

Resumen.—Los estudios de biodiversidad son necesarios para evaluar la diversidad de organismos a través de escalas espaciales y temporales. Estas evaluaciones pueden ser particularmente útiles para el monitoreo de cambios en la diversidad de organismos y la propagación de patógenos en respuesta al cambio climático. Los artrópodos vectores, como las garrapatas, son susceptibles a cambiar su rango geográfico con un clima más cálido, resultando potencialmente en la expansión de áreas de riesgo para las enfermedades transmitidas por vectores. Los datos de biodiversidad para el sur de Texas son deficientes, lo que es particularmente preocupante dada la abundancia de vida silvestre y ganado, ya que pueden ser importantes en el mantenimiento de poblaciones de garrapatas y patógenos. Se llevo a cabo un estudio de biodiversidad de pequeños mamíferos, garrapatas y patógenos transmitidos por garrapatas en el sur de Texas, combinando trabajo de campo, material de colecciones y técnicas moleculares. Registramos 19 especies de mamíferos pequeños y 3 especies de garrapatas y no detectamos patógenos transmitidos por garrapatas pertenecientes a los géneros *Borrelia* o *Rickettsia* en las garrapatas o mamíferos. Debido a la continua aparición de enfermedades transmitidas por garrapatas, recomendamos hacer colaboraciones con colecciones de museos y dueños de propiedades privadas interesados en la administración de terrenos. Esto permitirá que los investigadores desarrollen estudios para entender mejor la diversidad cambiante de mamíferos, garrapatas y patógenos con implicaciones en la salud humana y veterinaria en esta región de Texas.

An understanding and appreciation of biodiversity requires general knowledge of organismal diversity. Biodiversity surveys are some of the best ways to determine local diversity over time and space. Observations and records in natural history collections can provide some insight about how well known the flora and fauna are in a particular region and whether biodiversity surveys are needed. For example, Texas comprises 12 ecoregions

*Corresponding author: jessica.light@ag.tamu.edu

SAH orcid.org/0000-0002-4955-048X JEL orcid.org/0000-0001-6462-3045

TABLE 1. Land area and mammal records/observations from the southernmost counties in Texas in comparison to the whole of Texas. Data were obtained from iDigBio (www.idigbio.org) and iNaturalist (www.inaturalist.org) on 11 February 2021. Calculations are based on 106,162 preserved-specimen mammal records from iDigBio and 77,321 research-grade observations from iNaturalist. Only records with county information were included in the sample. The values in parentheses show the percent representation of each southern county within the total count of records from all Texas counties.

County	Land area (km^2)	iDigBio record count	iNaturalist observation count
Brooks	2440	79 (0.07%)	13 (0.02%)
Cameron	3300	3259 (3.07%)	756 (0.98%)
Hidalgo	4100	694 (0.65%)	1178 (1.52%)
Jim Hogg	2840	634 (0.6.%)	47 (0.06%)
Kenedy	5040	488 (0.46%)	130 (0.17%)
Starr	3180	183 (0.17%)	164 (0.21%)
Willacy	2030	1025 (0.97%)	102 (0.13%)
Zapata	2740	270 (0.25%)	53 (0.07%)
TOTAL (southernmost counties)	25,670 (3.69%)	6632 (6.25%)	2443 (3.16%)
TEXAS (all counties)	696,241	106,162	77,321

and a variety of habitat types across its 696,241-km^2 land area (Griffith et al. 2004). The large area and heterogeneity of these natural regions together provide habitats for over 160 species of native mammals (Schmidly and Bradley 2016). In South Texas, several distinct natural regions (e.g., Gulf Coastal Prairie and Marshes, Texas-Tamaulipan Thornscrub, and Coastal Sand Plains) provide unique and varied habitats for a variety of species. Out of all Texas mammal records, the percentage of mammal museum specimens originating from South Texas (6.25% of Texas mammal specimens on iDigBio are from South Texas; iDigBio accessed on 11 February 2021) and the percentage of mammal observations made in South Texas (3.16% of Texas mammal observations are from South Texas; iNaturalist accessed on 11 February 2021) is similar to the percentage of land area that South Texas makes up within the whole of the state (Table 1). However, the majority of the records from natural history collections are >50 years old. Furthermore, although iNaturalist records are relatively recent (this occurrence database was developed in 2008), most records are from the same geographic areas, usually ones that are easily accessible to a large number of community scientists (e.g., state parks). Thus, there is a lack of recent collections and observations of mammals in South Texas, indicating that our understanding of the biodiversity in this area is incomplete. Deficient mammalian biodiversity data from South Texas is problematic across several fronts, such as conservation and wildlife management, but most notably in understanding and monitoring of diseases vectored by hard ticks (Acari: Ixodidae) where both wildlife and livestock play a role in amplifying disease transmission (Tsao et al. 2021).

South Texas is currently an area of concern for some tick-borne diseases such as Texas cattle fever (also known as bovine babesiosis), the establishment and spread of which would have devastating impacts on the livestock industry. Although the hard ticks vectoring bovine babesiosis (cattle fever ticks *Rhipicephalus microplus* and *R. annulatus*) were eradicated from most of the United States in the mid-1940s, the ticks are still present in Mexico (where they, and the pathogen causing bovine babesiosis, are endemic) and along the Texas-Mexico border, resulting in a permanent quarantine zone between Texas and Mexico (Pérez de León et al. 2012, Giles et al. 2014). The movement of livestock from Mexico to Texas is carefully monitored to prevent cattle ticks from entering the United States; however, multiple studies have revealed that cattle fever ticks can parasitize common and free-ranging wildlife such as white-tailed deer (*Odocoileus virginianus*) and nilgai (*Boselaphus tragocamelus*) (e.g., Kistner and Hayes 1970, Pound et al. 2010, Lohmeyer et al. 2018, Olafson et al. 2018, Wang et al. 2020) and that wildlife can either show presence of, or antibodies to, bovine babesiosis (Ramos et al. 2010, García-Vázquez et al. 2015). Thus, an understanding of the wildlife-livestock interface is

imperative when trying to manage ticks and tick-borne diseases (e.g., Fèvre et al. 2006, Miller et al. 2013, Busch et al. 2014, Giles et al. 2014, Wiethoelter et al. 2015, Foley et al. 2017), especially in areas of concern such as South Texas.

A variety of other ixodid tick species belonging to the genera *Amblyomma*, *Dermacentor*, and *Ixodes* also occur in South Texas and are known to parasitize a large number of host species as well as vector multiple pathogens which can cause disease in humans, livestock, and wildlife (e.g., Sanders et al. 2008, Williamson et al. 2010, Shock et al. 2014, Medlin et al. 2015, Mitchell et al. 2016). Unlike cattle fever ticks, *Amblyomma*, *Dermacentor*, and *Ixodes* species employ a multihost life cycle in which more developed life stages (i.e., adults) feed on larger animals and less developed life stages (i.e., larvae and nymphs) feed on a variety of smaller animals such as rodents and birds. Rodents in particular play key roles as reservoirs for tick-borne pathogens worldwide (Kim et al. 2006, Meerburg et al. 2009, Mihalca and Sándor 2013, Busch et al. 2014). Diseases transmitted by hard ticks in the United States are most commonly caused by *Borrelia*, *Ehrlichia*, and *Rickettsia* pathogen species and other emerging viruses (e.g., Pritt et al. 2011, Stromdahl and Hickling 2012, Krause et al. 2015, Eisen et al. 2017, Paddock et al. 2017). Ticks and other vectors are susceptible to geographic range shifts with a warming climate, potentially resulting in the expansion of risk areas for vector-borne disease (e.g., Sonenshine 2018, Bede-Fazekas and Trájer 2019, Dehhaghi et al. 2019, Jones et al. 2019). In fact, changes in tick distributions are expected and already occurring with increasing climatic temperatures (Stromdahl and Hickling 2012, Feria-Arroyo et al. 2014, Kernif et al. 2016, Eisen et al. 2017, Nyangiwe et al. 2017, Sonenshine 2018). Thus, it is vital to document current distributions of reservoir hosts, vectors, and pathogens as a baseline for better understanding the effect of changing climate on vector-borne pathogens.

Previous research has noted that several rodent species distributed in South Texas are reservoir hosts for *Borrelia* and *Rickettsia* pathogens throughout the northeastern and midwestern United States (Gage et al. 1995, Stafford et al. 1999, Tanner et al. 2010) and eastern and southeastern United States (Levin et al. 1995, Oliver 1996, Magnarelli et al. 1999, Oliver et al. 2003, Rudenko et al. 2009). Although there are approximately 20 rodent species (Schmidly and Bradley 2016) that may act as potential hosts for tick species and reservoirs for pathogens in South Texas, it is generally unknown which, if any, South Texas rodents and ticks may be important in tick-borne pathogen cycles. Increasing both sampling and observations and establishing collaborations with landowners in South Texas are vital to understanding the dynamics of tick-borne disease cycles across the wildlife-livestock interface. Our objective was to gather data on the baseline diversity of small mammals (including their genetic diversity), on-host ticks, and tick-borne pathogens belonging to the genera *Borrelia* and *Rickettsia* throughout South Texas.

Methods

Study Area

Fieldwork was conducted year-round and opportunistically from June 2013 to June 2015 on 3 East Foundation (http://www.eastfoundation.net/) properties located within South Texas: El Sauz Ranch (ES), a 10,984-ha property located within southeastern Kenedy County and northwestern Willacy County (Gulf Prairies and Marshes and Coastal Sand Plains natural regions); San Antonio Viejo Ranch (SAV), a 60,033-ha property located within southern Jim Hogg County and northern Starr County (Texas Brush Country and Coastal Sand Plains natural regions); and Santa Rosa Ranch (SR), a 7544-ha property within Kenedy County (Coastal Sand Plains natural regions; Fig. 1). The East Foundation is an Agricultural Research Organization that owns over 215,000 acres of native rangelands in South Texas with a mission to promote the advancement of land stewardship through ranching, science, and education.

Sampling and Specimen Preparation

To capture a variety of small mammal species with differing life histories, we used 3 trapping techniques: (1) Sherman live trapping (H.B. Sherman Traps, Tallahassee, FL) for nocturnal small mammals, (2) Macabee humane kill trapping (Z.A. Macabee Gopher Trap Company, Los Gatos, CA) for fossorial

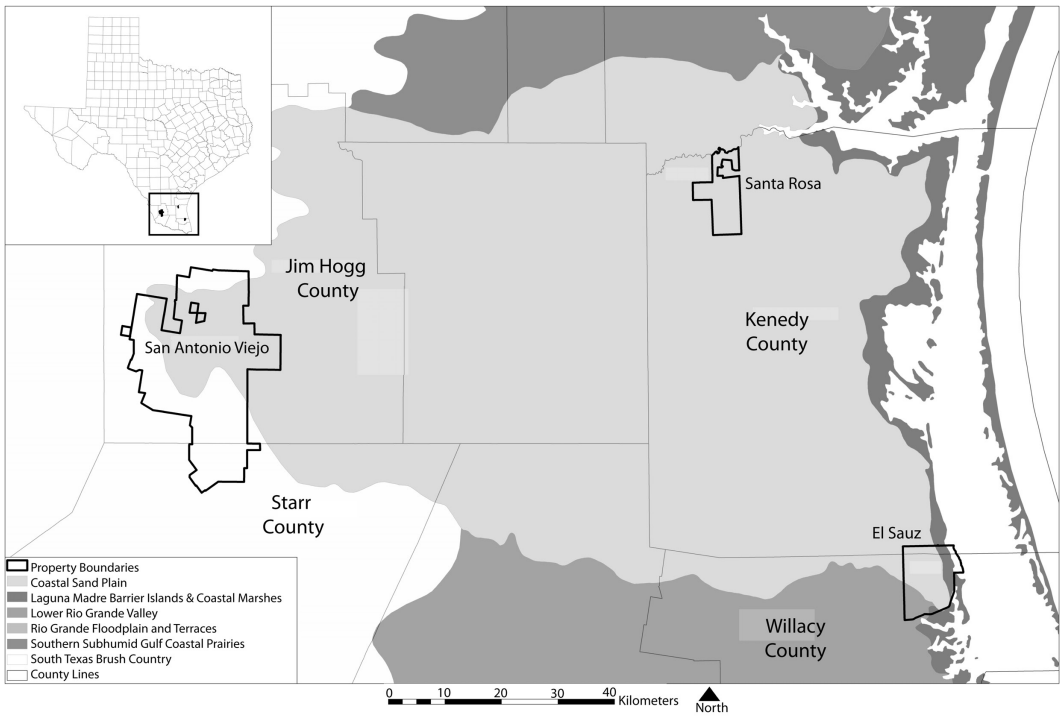

Fig. 1. Map of south Texas with East Foundation properties: El Sauz Ranch (10,984 ha in Kenedy and Willacy counties), San Antonio Viejo Ranch (60,033 ha in Jim Hogg and Starr counties), and Santa Rosa Ranch (7544 ha in Kenedy County). Shading indicates several Texas ecoregions in south Texas.

rodents, and (3) mist netting (Ecotone, Gdynia, Poland) for bats. We determined suitable trapping localities by scouting each property by vehicle and foot for diverse habitats to increase the biodiversity of species encountered. Field trips occurred throughout the year and were 3–20 days long. During field trips, efforts to capture small mammals occurred daily. To capture nocturnal and terrestrial small mammals, we set out approximately 240 Sherman live traps baited with sunflower seeds in transects of 40–80 traps, placed about 15 m apart every night at dusk. Traps were checked at sunrise, and small mammals were identified to species. For every trapping locality, a subset of randomly selected individuals of each species were euthanized for installation as voucher specimens into the Biodiversity Research and Teaching Collections at Texas A&M University (BRTC). Also included in our assessment were incidental trap mortalities from a separate study primarily conducted at SAV and ES from 2013 to 2016 (Baumgardt et al. 2019); these mortalities were deposited as scientific specimens at the BRTC.

We located active fossorial rodent (pocket gopher; *Geomys personatus*) mounds by surveying each property by foot following methods of Galán and Light (2017). Fresh mounds were uncovered with a shovel until tunnels were exposed. Once tunnels were exposed, we set humane (Macabee) kill traps which we checked every 15–20 min for up to 2 h. Since Macabee traps are kill traps, every pocket gopher captured was retained as a voucher specimen for the BRTC.

To capture bats, we set single-high and triple-high mist nets at dusk over water sources (e.g., livestock tanks) in a vector formation (2 mist nets set across water sources in a 60-degree angle to each other); we monitored these nets overnight. Up to 10 specimens per bat species per locality were retained as voucher specimens for the BRTC. All animals in this study were treated humanely according to the guidelines provided by the American Society of Mammalogists (Sikes et

al. 2016), the Texas A&M Animal Care and Use Committee (IACUC Animal Use Permits 2012-99 and 2015-0126), and Texas Parks and Wildlife Department Scientific Collecting Permit SPR-0409-082. All species collected were compared to Schimdly and Bradley (2016) to assess new county records, if present.

All retained specimens were frozen immediately after euthanization and until they could be processed at the BRTC. During preparation for installation into the BRTC, each collected mammal underwent a standardized protocol: identification of species and sex, collection of weight and body measurements, documentation of reproductive status, and thorough inspection for ectoparasites. Additionally, we obtained liver and kidney tissues from each specimen, and 2-mm-diameter ear biopsies were obtained from all specimens except bats, pocket gophers, and shrews. Tissues were stored in Nunc™ Cryovial collection tubes (Thermo Fisher Scientific, Waltham, MA) in a −80 °C freezer; ear biopsies and ectoparasites were stored in 70% ethanol and stored in a −20 °C freezer.

We removed ectoparasites by visual inspection, using forceps to remove each ectoparasite and vigorously brushing small mammal specimens over paper or aluminum foil. All ectoparasites were transferred to Nunc™ Cryovial tubes and stored in 70% ethanol in a −20 °C freezer. In the laboratory, ectoparasites were examined under both an Olympus SZX10 stereomicroscope and a Leitz Wetzlar compound light microscope, and then sorted categorically to fleas (Siphonaptera), lice (Phthiraptera), and ticks and mites (Acari). For the purpose of this study, only ticks were examined in greater detail using dichotomous keys to morphologically identify them to species (Sonenshine 1979, Keirans and Durden 1998, Sonenshine and Roe 2014). All other ectoparasites are held at the BRTC for future study.

Laboratory Methods

Laboratory work involved assessing small mammal genetic diversity across East Foundation properties, verifying tick morphological identifications, and testing for the presence of selected pathogens in small mammals and ticks. Tissue samples for mammal specimens used in the genetic analyses were obtained as loans from the BRTC. Total DNA was extracted from rodent liver tissue (25 mg), ticks (either whole nymphal individuals or pooled samples of larvae from the same host), and 2-mm-diameter ear biopsies using the E.Z.N.A. Tissue DNA extraction kit (Omega Bio-tek, Inc. Norcross, GA) according to manufacturer's recommendations except with a final elution of 60 μL of elution buffer at 70 °C for ticks and ear biopsies (Bunikis et al. 2004, Williamson et al. 2010).

We conducted a preliminary genetic assessment of South Texas small mammals by assessing the variation in a portion of the mitochondrial NADH dehydrogenase 2 (ND2) gene. Amplification and sequencing of this gene was performed using the primers L5219ND2 and H6313ND2 (Sorenson et al. 1999). Polymerase chain reaction amplifications (PCRs) were performed in 25-μL reaction volumes using 10 μL of Eppendorf HotMaster PCR Mix (Fisher Scientific, Pittsburg, PA), 1 μL of each primer (at 10 mM), and 1 μL of DNA template. Thermal-cycling parameters for ND2 required an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C (30 s), 50 °C (30 s), and 65 °C (90 s), and a final extension of 65 °C for 5 min. Annealing temperatures were lowered accordingly if specimens failed to amplify. PCR amplicons were visualized by gel electrophoresis; positive PCR reactions were purified using ExoSAP-IT (USB Corporation, Cleveland, OH); and all sequencing reactions were conducted at the Yale University DNA Analysis Facility on Science Hill (New Haven, CT) using ABI Prism BigDye Terminator cycle sequencing protocols (Applied Biosystems, Foster City, CA). Sequences were edited using Sequencher 4.9 (GeneCodes Corporation, Madison, WI), and primer sequences were removed and sequences were trimmed in reference to the translated protein sequence and aligned using Se-Al v2.01a11 (Rambaut 1996). Species identifications were verified with a BLAST search. We used PAUP* (Swofford 2003) to assess genetic divergences within and among East Foundation properties and TCS 1.21 (Clement et al. 2000) to conduct statistical parsimony analyses (Templeton et al. 1992) and construct haplotype networks for each species. TCS assembles the most parsimonious haplotype tree and estimates a 95% plausible set for all haplotype connections. Gaps were characterized as missing data, and

TABLE 2. Mammal species retained from East Foundation properties El Sauz (ES), San Antonio Viejo (SAV), and Santa Rosa (SR) from June 2013 to June 2015. Mammal species are organized by order and family, and all mammal taxonomy follows Schmidly and Bradley (2016) with the exception of the Mexican spiny pocket mouse, which is placed in the genus *Heteromys*. Number of individuals per species retained is indicated per property; numbers of incidental mortalities from Baumgardt et al. (2019) are not included.

Mammal species	ES	SAV	SR	Total
Order Chiroptera: Vespertilionidae				
Dasypterus intermedius (Northern yellow bat)	0	1	0	1
Nycticeius humeralis (Evening bat)	9	2	8	19
Order Rodentia: Cricetidae				
Baiomys taylori (Northern pygmy mouse)	10	0	1	11
Neotoma micropus (Southern plains woodrat)	1	3	0	4
Onychomys leucogaster (Northern grasshopper mouse)	8	4	10	22
Oryzomys texensis (Texas marsh rice rat)	1	0	0	1
Peromyscus leucopus (White-footed deermouse)	54	18	16	88
Reithrodontomys fulvescens (Fulvous harvest mouse)	6	0	1	7
Sigmodon hispidus (Hispid cotton rat)	12	12	13	37
Order Rodentia: Geomyidae				
Geomys personatus (Texas pocket gopher)	5	5	1	11
Order Rodentia: Heteromyidae				
Chaetodipus hispidus (Hispid pocket mouse)	16	17	34	67
Dipodomys compactus (Gulf coast kangaroo rat)	5	2	6	13
Dipodomys ordii (Ord's kangaroo rat)	0	1	0	1
Heteromys irroratus (Mexican spiny pocket mouse)	3	0	0	3
Perognathus merriami (Merriam's pocket mouse)	4	16	4	24
Order Rodentia: Sciuridae				
Ictidomys parvidens (Rio Grande ground squirrel)	0	1	0	1
Xerospermophilus spilosoma (Spotted ground squirrel)	0	0	1	1
Order Soricomorpha: Soricidae				
Cryptotis parva (Least shrew)	1	0	1	2
Notiosorex crawfordi (Crawford's desert shrew)	1	0	0	1
TOTAL	136	82	96	314

linkages between taxa represent mutational events.

We confirmed tick morphological identifications via PCR of a fragment (ca. 360 base pairs [bp]) of the mitochondrial 12S rDNA gene following protocols developed by Beati and Keirans (2001). If ticks could not be identified using 12S, a fragment (ca. 410 bp) of the mitochondrial 16S rDNA gene was amplified following Mangold et al. (1998). Methods for tick PCR clean-up, sequencing, sequence annotation, and GenBank BLAST searches were the same as described above.

A random subset of ear biopsies and ticks representing multiple host species were subjected to *Borrelia* and *Rickettsia* pathogen screening. *Borrelia* species were amplified using a nested PCR for the 16S–23S rRNA intergenic spacer region (IGS; primers: IGS-F, IGS-R, IGS-Fn, and IGS-Rn) following protocols developed by Bunikis et al. (2004) for a final product of approximately 900 bp for Lyme group *Borrelia* and 500 bp for relapsing fever *Borrelia*. *Rickettsia* species were amplified by screening samples using a traditional PCR protocol targeting 617 bp of the citrate synthase (gltA) gene using primers (RrCS 372 and RrCS 989) and protocols developed by Williamson et al. (2010). Included in each PCR, respectively, were *Borrelia* and *Rickettsia* positive controls obtained from field-collected ticks previously determined to be positive for each pathogen (*A. maculatum* collected from Attwater Prairie Chicken National Wildlife Refuge in Texas from Castellanos et al. 2016 and *I. scapularis* collected from the midwestern United States, respectively). We used the same methods described above for pathogen PCR clean-up, sequencing, sequence annotation, and GenBank BLAST searches.

RESULTS

All 3 field surveys resulted in the collection and retention of 314 small mammal specimens representing 3 orders, 6 families, and 19 species (Table 2, Supplementary Material 1).

Table 3. Total number of small mammal individuals retained per species from June 2013 to June 2015 per county. Asterisks (*) denote new county records. All mammal taxonomy follows Schmidly and Bradley (2016) with the exception of the Mexican spiny pocket mouse, which is placed in the genus *Heteromys*. Common names are given in Table 2. Numbers of incidental mortalities from Baumgardt et al. (2019) are not included.

Mammal species	Jim Hogg	Kenedy	Starr	Willacy	Total
Order Chiroptera: Vespertilionidae					
Dasypterus intermedius	1*	0	0	0	1
Nycticeius humeralis	2*	17	0	0	19
Order Rodentia: Cricetidae					
Baiomys taylori	0	2	0	9	11
Neotoma micropus	0	1	3	0	4
Onychomys leucogaster	2	15	2	3	22
Oryzomys texensis	0	1	0	0	1
Peromyscus leucopus	13	53	5	17	88
Reithrodontomys fulvescens	0	5	0	2	7
Sigmodon hispidus	0	19	12	6	37
Order Rodentia: Geomyidae					
Geomys personatus	5	2	0	4	11
Order Rodentia: Heteromyidae					
Chaetodipus hispidus	11	43	6	7	67
Dipodomys compactus	2	10	0	1	13
Dipodomys ordii	1	0	0	0	1
Heteromys irroratus	0	2	0	1	3
Perognathus merriami	11	6	5	2*	24
Order Rodentia: Sciuridae					
Ictidomys parvidens	1	0	0	0	1
Xerospermophilus spilosoma	0	1	0	0	1
Order Soricomorpha: Soricidae					
Cryptotis parva	0	2*	0	0	2
Notiosorex crawfordi	0	1*	0	0	1
Total	49	180	33	52	314

Sherman trapping resulted in the retention of 283 small terrestrial mammals (14 rodent species and 2 shrew species); trapping for fossorial mammals resulted in the capture of 11 pocket gopher individuals belonging to 1 species; and mist-netting for bats resulted in the retention of 20 bats representing 2 species (Table 2, Supplementary Material 1). Incidental trap mortalities from Baumgardt et al. (2019) resulted in additional specimens across the East Foundation properties, but no additional species.

Species richness at SAV was highest with 15 species (ES and SR each had 12 species), with some species only captured at one East Foundation property (Table 2). For example, *Oryzomys texensis*, *Heteromys irroratus*, and *Notiosorex crawfordi* were only collected at SAV (Table 2). The species richness of small mammals captured was highest in Kenedy County and lowest in Starr County (Table 3). Our collection efforts resulted in new county records for *Dasypterus intermedius* and *Nycticeius humeralis* (Jim Hogg County), *Perognathus merriami* (Willacy County), and *Cryptotis parva* and *Notiosorex crawfordi* (Kenedy County; Table 3). The most abundant terrestrial small mammal species captured and retained were rodents from the families Cricetidae and Heteromyidae (Tables 2, 3; Supplementary Material 1): *Peromyscus leucopus* (n = 88), *Sigmodon hispidus* (n = 37), and *Onychomys leucogaster* (n = 22) in the former and *Chaetodipus hispidus* (n = 67) and *Perognathus merriami* (n = 24) in the latter. The most abundant bat species collected was the evening bat (*Nycticeius humeralis*; n = 19).

We successfully amplified and sequenced ND2 in 11 small mammal species distributed across the 3 East Foundation properties (Supplementary Material 1). We were not able to assess all collected species due to small sample sizes (Supplementary Material 1). In general, genetic variation within and among populations was small, ranging from 0.26% to 1.12% uncorrected p distances within properties and 0.23% to 1.32% among properties (Table 4). Within species, haplotypes often were shared among East Foundation properties; no differentiation by East

Table 4. Average uncorrected *p*-distances for the ND2 gene across 11 species of small mammals and the 3 East Foundation properties El Sauz (ES), San Antonio Viejo (SAV), and Santa Rosa (SR). Number of base pairs (bp) examined is listed to the right of each species name. Missing values indicate that no individuals, or only 1 or 2 individuals, were assessed at a particular property (Supplementary Material 1). All mammal taxonomy follows Schmidly and Bradley (2016), and common names are given in Table 2. Specific localities of specimens used in the phylogeographic analysis are listed in Supplementary Material 1.

Mammal species	Within ES	Within SAV	Within SR	ES vs. SAV	ES vs. SR	SAV vs. SR
Order Chiroptera: Vespertilionidae						
Nycticeius humeralis (969 bp)	0.73%	—	0.56%	0.89%	0.62%	1.32%
Order Rodentia: Cricetidae						
Baiomys taylori (981 bp)	0.46%	—	—	—	0.23%	—
Neotoma micropus (927 bp)	—	0.26%	—	0.24%	—	—
Onychomys leucogaster (978 bp)	0.41%	0.47%	0.55%	0.43%	0.49%	0.49%
Peromyscus leucopus (1014 bp)	0.89%	1.12%	0.93%	0.94%	0.94%	0.91%
Reithrodontomys fulvescens (990 bp)	0.61%	—	—	0.63%	0.57%	1.11%
Sigmodon hispidus (933 bp)	0.32%	0.59%	0.52%	0.43%	0.42%	0.56%
Order Rodentia: Geomyidae						
Geomys personatus (990 bp)	1.0%	0.67%	—	0.84%	—	—
Order Rodentia: Heteromyidae						
Chaetodipus hispidus (993 bp)	0.50%	0.42%	0.58%	0.51%	0.61%	0.51%
Dipodomys compactus (1005 bp)	0.63%	0.60%	0.78%	0.61%	0.70%	0.63%
Perognathus merriami (960 bp)	0.78%	0.58%	0.76%	0.64%	0.66%	0.62%

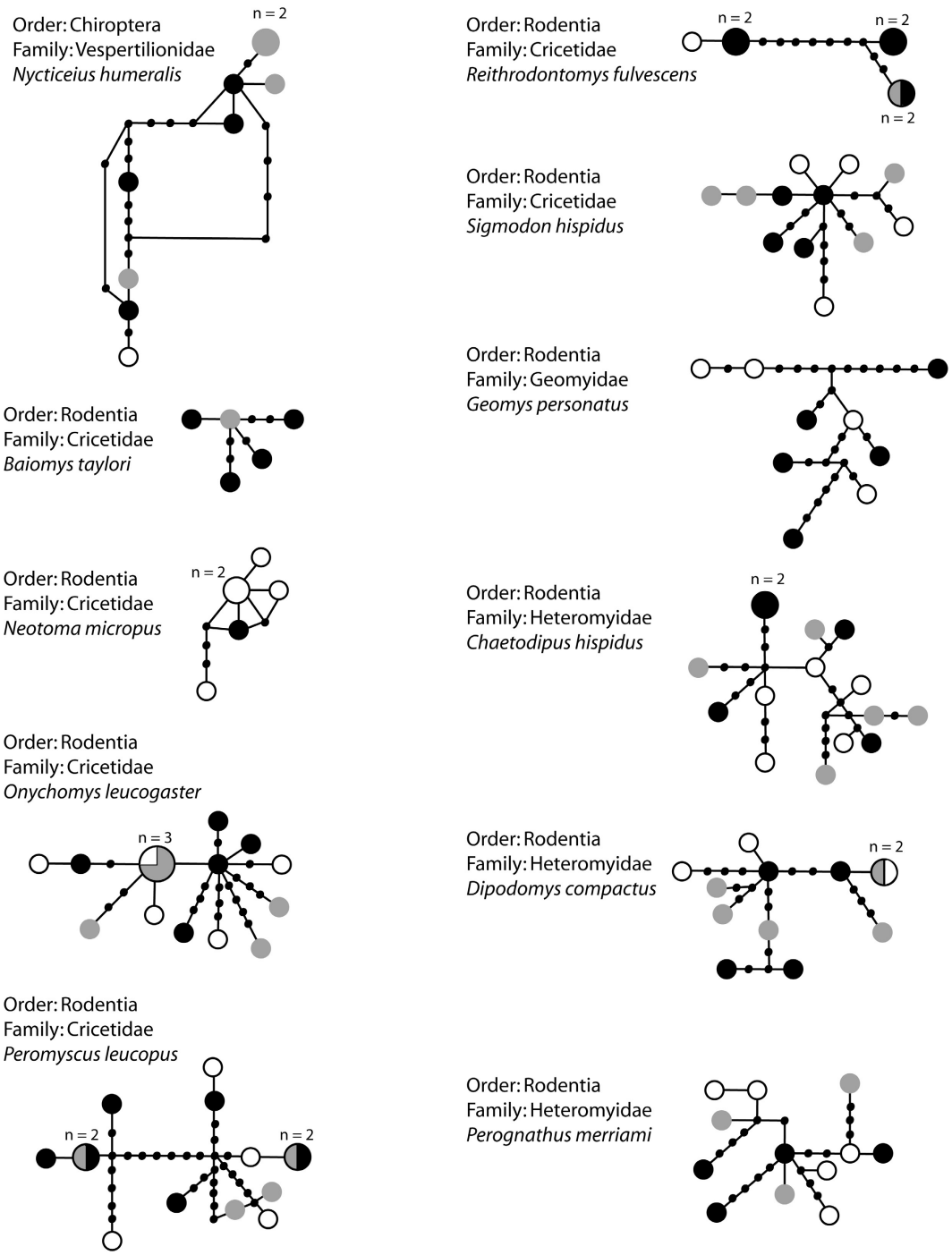

Fig. 2. Statistical parsimony haplotype networks based on the mitochondrial ND2 gene for 11 small mammal species distributed across south Texas. Observed haplotypes are shown as large circles, with haplotype frequencies >1 indicated. Connections between haplotypes represent single mutations, with inferred haplotypes denoted by small black circles. Haplotype shading corresponds to East Foundation property: El Sauz Ranch (black shading), San Antonio Viejo (white shading), and Santa Rosa Ranch (gray shading).

Table 5. Small mammals (organized by order, family, and species) infested with tick species *Amblyomma maculatum*, *Dermacentor variabilis*, and *Ixodes woodi*. Tick life stage (larva or nymph) also is indicated. The number of infested small mammals is represented as a whole fraction (with percent infestation in parentheses). Superscripts correspond to East Foundation properties where on-host ticks were found: (a) El Sauz, (b) San Antonio Viejo, and (c) Santa Rosa. Mammal common names are given in Table 1. Specific host individuals parasitized by ticks are listed in Supplementary Material 1.

		A. maculatum		*D. variabilis*		*I. woodi*	
Host species	Infested with ticks	Larva	Nymph	Larva	Nymph	Larva	Nymph
Order Chiroptera: Vespertilionidae							
Dasypterus intermedius	0/1 (0%)						
Nycticeius humeralis	0/19 (0%)						
Order Rodentia: Cricetidae							
Baiomys taylori	1/11 (9.1%)	0	0	2[a]	0	0	0
Neotoma micropus	0/8 (0%)						
Onychomys leucogaster	7/43 (16.3%)	1[a]	0	1[a], 6[b]	1[b]	6[b]	0
Oryzomys texensis	0/1 (0%)						
Peromyscus leucopus	23/140 (16.4%)	0	1[a]	24[a], 12[b], 1[c]	8[a], 10[b], 6[c]	7[b]	0
Reithrodontomys fulvescens	0/8 (0%)						
Sigmodon hispidus	2/41 (4.9%)	0	0	5[a]	0	0	0
Order Rodentia: Geomyidae							
Geomys personatus	0/11 (0%)						
Order Rodentia: Heteromyidae							
Chaetodipus hispidus	1/75 (1.3%)	0	2[a]	0	0	0	0
Dipodomys compactus	0/16 (0%)						
Dipodomys ordii	0/1 (0%)						
Heteromys irroratus	0/3 (0%)						
Perognathus merriami	0/38 (0%)						
Order Rodentia: Sciuridae							
Ictidomys parvidens	0/2 (0%)						
Xerospermophilus spilosoma	0/1 (0%)						
Order Soricomorpha: Soricidae							
Cryptotis parva	0/2 (0%)						
Notiosorex crawfordi	0/1 (0%)						
Total	34/422 (8.1%)	1 (1[a])	3 (3[a])	51 (32[a], 18[b], 1[c])	25 (8[a], 11[b], 6[c])	13 (13[b])	0

[a]El Sauz
[b]San Antonio Viejo
[c]Santa Rosa

Foundation property was noted (Fig. 2). All ND2 sequences were submitted to GenBank (GenBank numbers MW843662–MW843778; Supplementary Material 1).

Ectoparasites were found on many of the sampled small mammal specimens. Here, we report only hard-tick specimens from which we were able to confirm identification using molecular markers (see below). Of the 422 small mammal specimens examined for hard ticks (314 specimens from our collecting efforts and 108 specimens from Baumgardt et al. [2019]; Supplementary Material 1), 34 were parasitized for an overall tick infestation prevalence of 8.1% (Table 5, Supplementary Material 1). The 34 individuals infested with ticks represented 5 rodent species: the cricetids *Baiomys taylori*, *Onychomys leucogaster*, *Peromyscus leucopus*, and *Sigmodon hispidus* and the heteromyid *Chaetodipus hispidus* (Table 5). *Peromyscus leucopus* had the highest infestation prevalence (16.4%; with on-host ticks found across all 3 study areas; Table 5, Supplementary Material 1), followed by *Onychomys leucogaster* (16.3%; with on-host ticks present only at ES and SAV; Table 5, Supplementary Material 1). Of the small mammal species that were parasitized by ticks, *Chaetodipus hispidus* had the lowest overall tick infestations of 1.3%, with on-host ticks present only at ES (Table 5, Supplementary Material 1). All other on-host ticks occurred only at ES.

In total, 93 hard ticks (all larvae and nymphs) were collected from rodents across all 3 properties (Table 5, Supplementary Material 1). The engorged status and missing mouthparts in several of the larvae complicated the ability to identify the specimens based on morphologic features. Thus, we relied on our molecular work to determine tick species identifications. The 93 ticks were divided into 51 molecular samples: 28 individual nymph samples and 23 larval pools (representing a total of 64 larvae). Larval pools consisted of 1–11 larval ticks, all from the same host individual, homogenized together to make a DNA sample. Molecular identifications determined the presence of 3 tick species (Table 5): *Amblyomma maculatum* (Gulf coast tick; $n = 4$, 1 larvae and 3 nymphs), *Dermacentor variabilis* (American dog tick; $n = 76$, 51 larvae across 19 larval pools and 25 nymphs), and *Ixodes woodi* ($n = 13$ larvae across 3 larval pools). *Dermacentor variabilis* was the most prevalent tick species, parasitizing 4 small mammal species, and was encountered most frequently at ES (Table 5). *Amblyomma maculatum* was encountered at ES and parasitized *Chaetodipus hispidus*, *Onychomys leucogaster*, and *Peromyscus leucopus* (Table 5). *Ixodes woodi* ticks were found on *Onychomys leucogaster* and *Peromyscus leucopus* at SAV (Table 5). Ticks were collected in near equal numbers from ES ($n = 44$) and SAV ($n = 42$), with 7 ticks collected from SR (Table 5). We also note that 2 additional soft ticks (*Carios* sp.) were collected from *Peromyscus leucopus* from SAV (data available upon request; Supplementary Material 1). Sixteen additional larval and nymphal ticks also were collected from 9 hosts (1 *Chaetodipus hispidus*, 3 *Onchyomys leucogaster*, and 5 *Peromyscus leucopus* from SAV and ES); however, we were not able to confidently identify these ticks to species. All tick sequences were submitted to GenBank (GenBank accessions OK393964–OK394020).

Ear biopsies from a subset of our collections were screened for *Borrelia* and *Rickettsia* pathogen species. In total, we screened 271 small mammal specimens (Supplementary Material 1), and all were negative for both *Borrelia* and *Rickettsia* species. All tick DNA samples ($n = 61$) were also screened for *Borrelia* and *Rickettsia* species, and all yielded negative results for both genera.

Discussion

This baseline biodiversity survey gives important insight into small mammal and tick diversity across Jim Hogg, Kenedy, Starr, and Willacy counties in South Texas. We encountered 19 small mammal species, 3 ixodid tick species, and no *Borrelia* or *Rickettsia* species. Future biodiversity surveys may expand upon our findings with the detection of additional small mammal, tick, or pathogen species. Alternatively, additional work may reveal that distributions of some species have shifted or contracted. This, and other, baseline biodiversity surveys are necessary to assess organismal diversity across spatial and temporal scales, and they can be particularly useful for monitoring changes in organismal diversity and disease spread in response to landscape use and climate change.

Collections-based research was an important component of this South Texas biodiversity assessment. Scientific collections provide innumerable benefits to education and scientific research. These collections house millions of specimens, historical images, documents, and other materials that are invaluable sources of primary data for researchers working in a variety of fields (Suarez and Tsutsui 2004, Mares 2009, Kemp 2015, Cook and Light 2019, Miller et al. 2020). Importantly, the future research potential of museum specimens remains to be realized, especially given recent advancements in technology (McLean et al. 2016, Cook and Light 2019). Thus, it is imperative to continue to collect and install specimens into natural history collections (Rocha et al. 2014, Hope et al. 2018). In reference to diseases, museum specimens have been used successfully to track pathogens and contaminants over time (e.g., Yates et al. 2002, Tsangaras and Greenwood 2012, Ávila-Arcos et al. 2013, DiEuliis et al. 2016, Tiee et al. 2018). Natural history collections are therefore vital repositories that document past organismal (including pathogen) biodiversity and provide baseline materials necessary to forecast species distributions, extinction risk, and disease spread (Newbold 2010, Schindel and Cook 2018). Notably, there have been several recent calls for collaboration with natural history collections (including vouchering specimens) to help understand, survey, and mitigate emerging and infectious pathogens, parasites, and diseases (Dunnum et al. 2017, McLean et al. 2019, Cook et al. 2020, Thompson et al. 2021), which we hope is exactly what our study has done.

In the case of mammals, our biodiversity survey resulted in new county records for 5 mammal species: *Dasypterus intermedius* (northern yellow bat; Jim Hogg County), *Nycticeius humeralis* (evening bat; Jim Hogg County), *Perognathus merriami* (Merriam's pocket mouse; Willacy County), *Cryptotis parva* (least shrew; Kenedy County), and *Notiosorex crawfordi* (Crawford's desert shrew; Kenedy County; Table 3). None of these new county records are outside the known geographic ranges of each species (Schmidly and Bradley 2016), providing support that the distributions of these species do indeed encompass these counties. Importantly, our work has resulted in the availability of genetic resources for all of the mammal species we collected from across South Texas. These resources were rarely available before our baseline survey, as many specimens in natural history collections were most recently collected prior to when it became standard to take tissues for genetic work. These genetic resources can facilitate untold future research, including surveys for contaminants and disease as well as examination of genetic variation over time and space. In our case, genetic variation within each species across our study sites was low (Table 4, Fig. 2). Although preliminary, our assessment of genetic variation across species, our study sites, and South Texas counties can be informative for the design of future population genetics studies in this region. Overall, our findings highlight the importance of continuing biodiversity surveys to confirm species presence (as well as abundance) and distributions over geographic regions as well as the need for deposition of specimens (along with associated data, tissues, parasites, etc.) in natural history collections for future research.

Only 8.1% of the small mammal specimens examined were parasitized with ticks that we were able to identify to species; additional screening of host specimens paired with molecular methods to confidently identify ticks will likely result in a higher tick prevalence. In total, we encountered 3 ixodid tick species: *Amblyomma maculatum*, *Dermacentor variabilis*, and *Ixodes woodi* (Table 5). *Amblyomma maculatum* and *D. variabilis* are 2 commonly encountered tick species in Texas (Teel et al. 2010, Williamson et al. 2010, Mitchell et al. 2016) and were common parasites of the small mammals examined in this study (Table 5). *Amblyomma maculatum* is known to transmit a variety of *Rickettsia* pathogens, including *Rickettsia parkeri*, the causative agent for Rocky Mountain spotted fever and tick paralysis (e.g., Parola and Raoult 2001, Williamson et al. 2010, Trout Fryxell et al. 2015, Castellanos et al. 2016, Mays et al. 2016, Mitchell et al. 2016). *Dermacentor variabilis* can vector *R. rickettsii* (another causative agent of Rocky Mountain spotted fever) and may play a role in transmitting tularemia and other pathogens (Williamson et al. 2010, Stromdahl et al. 2011, Trout Fryxell et al. 2015). *Ixodes woodi* has been found on South Texas and Mexican mammals in the past (Guzmán-Cornejo et al.

2007, Charles et al. 2012), and it is known to harbor and potentially transmit *Rickettsia*-like bacteria (Kurtti et al. 2002, Leclerque and Kleespies 2012). Given that other tick species are known from South Texas (e.g., Olafson et al. 2020), it is possible that future research may uncover additional tick diversity.

Although we did not detect the presence of *Borrelia* or *Rickettsia* pathogens in any tick or mammal specimens examined, that does not necessarily mean that pathogens are not present in South Texas ticks or small mammals. Previous work has found low pathogen prevalence in Texas mammals (Castellanos et al. 2016) as well as across broad national surveys (Nieto et al. 2018, Ginsberg et al. 2021). Given low prevalence, larger sample sizes for both small mammals and ticks may be necessary for confidence in pathogen prevalence estimates. Importantly, a variety of other tick-borne pathogens in addition to those caused by *Borrelia* or *Rickettsia* species may be present in South Texas (Rar and Golovljova 2011), any of which may be emerging in livestock or humans especially given expected changes to tick distributions with climate change (Stromdahl and Hickling 2012, Kernif et al. 2016, Eisen et al. 2017). Future studies in this region should screen for all potential pathogens.

Our work serves as a baseline for future small mammal, tick, and tick-borne pathogen diversity assessments in South Texas. Future work across South Texas can expand from here by sampling small mammals and ticks from a wide variety of habitats using multiple capture techniques across time and by examining larger sample sizes as well as a larger suite of potentially emerging pathogens. Working with natural history collections to deposit specimens will only strengthen the ability of researchers to develop a better understanding of small mammal and tick diversity and aid in determining risk assessment to human and veterinary health from this region of Texas in an era of increasing disease risk due to emerging pathogens.

Supplementary Material

One online-only supplementary file accompanies this article (https://scholarsarchive.byu.edu/wnan/vol82/iss2/4).

Supplementary Material 1. Specimens examined. All specimens are housed at the Biodiversity Research and Teaching Collections at Texas A&M University and are organized by catalog number. Specimen taxonomy, sex, collection data and locality, and collector information are given.

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