

UPDATED DISTRIBUTIONS OF THREE SPECIES OF YELLOW BAT (*DASYPTERUS*) IN TEXAS BASED ON SPECIMEN RECORDS

**SYDNEY K. DECKER^{1,2,5}, DIANNA M. KREJSA¹, LARAMIE L. LINDSEY^{1,3},
RICHARD P. AMOATENG^{1,4}, AND LOREN K. AMMERMAN¹**

¹Department of Biology, Angelo State University, ASU Station 10890, San Angelo, Texas 76909, USA

²Department of Evolution, Ecology, and Organismal Biology, Ohio State University, 318 West 12th Avenue,
Columbus, Ohio 43210, USA

³Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue,
Saint Paul, Minnesota 55108, USA

⁴Connolly Hospital Blanchardstown, Mill Road, Abbotstown, Dublin, D15 X40D, Ireland

⁵Corresponding author; e-mail: decker.391@osu.edu

Abstract.—Updating species ranges and documenting range extensions with new county records helps monitor the status of populations, provides insight into life-history traits, and informs conservation decisions. Three species of yellow bats (*Dasypterus* spp.) reach the limits of their distribution in Texas and have been documented to be expanding their previously known range. Here, we updated the distribution maps of the three species of yellow bats (*D. ega*, *D. intermedius*, and *D. xanthinus*) in Texas using vouchered specimens. We confirmed species identities using morphological measurements and, in some cases, sequence from a fragment of the mitochondrial cytochrome *b* gene (*Cytb*). We documented eight county records for *D. ega*, eight county records for *D. intermedius*, and one county record for *D. xanthinus* in Texas. Updated distribution maps produced using county records help refine our understanding of the natural history of these species.

Key Words.—Chiroptera; cytochrome *b*; *Lasiurus*; range extension

INTRODUCTION

Yellow bats are medium-large vespertilionid bats, are insectivorous, roost in trees, and are found in North, Central, and South America. Three species of yellow bats occur in the U.S.: Southern Yellow Bats (*Dasypterus ega*), Northern Yellow Bats (*D. intermedius*), and Western Yellow Bats (*D. xanthinus*). Yellow bats were formerly included in the genus *Lasiurus*, until reassigned to *Dasypterus* by Baird et al. (2015) based on genetic distance and divergence times between yellow bats and other *Lasiurus* bats. Additionally, Western Yellow Bats were originally named as a subspecies of Southern Yellow Bats (*L. ega xanthinus*) until elevated to species level in 1988 (Baker et al. 1988).

Migration in these species may be facultative or absent, as individuals from portions of their respective ranges have been demonstrated to be present year-round (Baker et al. 1971; Zabriskie et al. 2019). Litter size in Western Yellow Bats is typically two, whereas in Southern and Northern Yellow Bats the litter size is 2–4 (Ammerman et al. 2012). Yellow bats roost primarily in the dried fronds of palm trees such as Mexican Fan Palms (*Washingtonia robusta*) and Texas Sabal Palms (*Sabal mexicana*) in Texas (Chapman and Chapman 1990; Jimenez 2016). Northern Yellow Bats also roost in Spanish Moss (*Tillandsia usneoides*) associated with hardwood trees in the southeastern U.S. (Constantine 1958; Coleman et al. 2012), and Western Yellow Bats have been documented roosting in Dagger Yucca (*Yucca carnerosana*) in Big Bend National Park (Higginbotham et al. 2000) and deciduous trees, such

as sycamore (*Platanus* spp.) and cottonwood (*Populus* spp.; Bond 1970).

Southern Yellow Bats are medium-sized (forearm length: 42–48 mm), have yellow-brown pelage, and are distributed from south Texas to Argentina (Esbérard and Moreira 2006; Ammerman et al. 2012). Northern Yellow Bats are the largest of the yellow bats (forearm length: 45–56 mm) with yellow-brown pelage and are distributed from South Carolina in the U.S. to northern Nicaragua, primarily inhabiting coastal regions (Webster et al. 1980; Ammerman et al. 2012). Western Yellow Bats are of a similar size to Southern Yellow Bats (forearm length: 43–47 mm), have pale yellow pelage, and occur in southwestern U.S. and northeastern Mexico (Ammerman et al. 2012). The three species are difficult to distinguish from one another morphologically, especially as juveniles, and require consideration of range as well as genetic techniques to confirm identification to species level (Baker et al. 1988; Morales and Bickham 1995; Tipps et al. 2011).

In the U.S., each of the three species of yellow bats reach limits of their distributions in Texas. Southern Yellow Bats meet the northern limit of their range in Texas and are historically only known from the southernmost counties of Texas. Texas is the westernmost limit of the range of Northern Yellow Bats, which are primarily a coastal species; in Texas their range follows the coast of the Gulf of Mexico. Western Yellow Bats were first documented in Texas in 1996 in Big Bend National Park, Brewster County (Higginbotham et al. 1999). Since this first record, the species has been documented in three additional Texas counties and appears to be restricted to

western Texas, the easternmost limit of their range.

The range of Southern Yellow Bats has recently extended as reported by Demere et al. (2012) with an extension of the northern boundary into Fayette and Comal counties. Demere et al. (2012) also reported extension of Northern Yellow Bats into Webb, Frio, Uvalde, and Starr counties, representing extension of the western range boundary. Tipps et al. (2011) reported extension of Western Yellow Bats into El Paso County; however, these specimens tested positive for the rabies virus and therefore may have exhibited abnormal behavior. Additional specimens are important to confirm the presence of Western Yellow Bats in El Paso County. Range extension for Western Yellow Bats has also recently been reported in the Chihuahuan Desert Ecoregion of New Mexico and a new record from Las Cruces, New Mexico, represents a northeastward range extension of approximately 210 km in the state (Zabriskie et al. 2019). Yellow bats are all considered to be rare across their respective ranges and thus, much is unknown about their life-history traits and range limits. The objective of this study was to update the distribution of the three species of yellow bats in Texas based on current occurrence records.

METHODS

Yellow bats that we examined were submitted to the Texas Department of State Health Services (DSHS) in Austin, Texas, between 2008 and 2019 for rabies testing. Of those submitted during this time period, approximately 760 yellow bats tested negative for the rabies virus, were frozen, and were added into a database that was used to identify new county records. We determined specimens to be new county records if we did not find a museum voucher specimen for a species from the county. We used distribution maps in Ammerman et al. (2012), lists of specimens examined on the website for Bats of Texas (Ammerman et al. 2012; www.batsoftexas.com), and new county record papers (Demere et al. 2012, Tipps et al. 2011) to determine records of interest. Some counties had only literature records or Texas DSHS records, indicating that a specimen was recorded in the county but no voucher specimen was identified. Texas DSHS records are generally comprised of specimens that were incinerated after being recorded in the database and thus no collections-based voucher specimen exists. Specimens were incinerated if they tested positive for rabies or if they were not deposited at another institution.

Voucher specimens were deposited at the Angelo State Natural History Collections (ASNHC) or the Natural Science Research Laboratory at Texas Tech University (NSRL-TTU) and were prepared as skull only, study skin, or fluid preserved specimens. Fluid specimens were preserved in 70% ethanol after fixation in 10% formalin. The county of collection and the date of collection of the specimen by Texas DSHS were recorded for each

specimen. More specific collection data, such as specific locality or information about collectors, was unavailable due to data privacy restrictions set by the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Age (adult or juvenile) and sex were determined and recorded for each specimen and standard morphological measurements (total length, tail length, hindfoot length, ear length, length of the tragus, and forearm length) were taken and recorded. We used measurements and other key characteristics as described in Ammerman et al. (2012) to confirm species identity. Tissue samples (heart, kidney, liver, spleen, lung, and muscle) were taken when possible, frozen, and deposited at the ASNHC (issued an ASK number).

Due to the morphological similarities of the species of *Dasypterus*, we confirmed the species identity of some specimens using DNA sequence data from the mitochondrial cytochrome *b* (*Cytb*) gene, commonly used for species identification by DNA barcoding in mammals (Kocher et al. 1989; Irwin et al. 1991). We extracted DNA from frozen tissues (heart, kidney, or liver) according to the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California) protocols. We visually assessed the quality of the DNA using gel electrophoresis (0.8% agarose in 1× sodium borate solution) and quantified for DNA yield and purity using a NanoDrop Lite (Thermo Fisher Scientific Inc., Waltham, Massachusetts) spectrophotometer or a Qubit 1.0 fluorometer (Invitrogen Corp., Carlsbad, California). We amplified *Cytb* using polymerase chain reaction (PCR) primer sets MVZ05/MVZ04 (Smith and Patton 1991) or LGL765/LGL766 (Bickham et al. 1995). Amplifications were done using 1× standard Taq reaction buffer (New England BioLabs, Ipswich, Massachusetts), 1 unit of Taq polymerase (New England BioLabs), 2.0 mM MgCl₂ (New England BioLabs), 0.2 mM of each dinucleoside triphosphate (Thermo Fisher Scientific Inc.), 0.16 μM of each forward and reverse primer, 50–500 ng of template DNA, and RNase free water as needed to meet a final reaction volume of 12.5 μL.

The thermal profile for the MVZ05/MVZ04 primer set began with a denaturing temperature of 94° C for 2 min, followed by 39 cycles of 94° C for 1 min, 50° C or 48° C for 1 min, 72° C for 1 min, followed by a final extension of 72° C for 5 min. The thermal profile for the LGL765/LGL766 primer set had an initial denaturing step of 94° C for 2 min, followed by 35 cycles of 92° C for 1 min, annealing at 52° C for 1 min, 72° C for 1 min, and a final extension of 72° C for 5 min. We verified product amplification with gel electrophoresis (0.8% or 1% agarose in 1× sodium borate solution). We then purified PCR products to prepare for sequencing with ExoSAP-IT PCR Product Cleaning Reagent (Thermo Fisher Sci.) following the protocol of the manufacturer. For the MVZ05/MVZ04 primer set, we sequenced the purified samples following the protocol of the manufacturer with the GenomeLab DTCS-Quick Start Kit in a Beckman Coulter CEQ 8000 Genetic Analysis

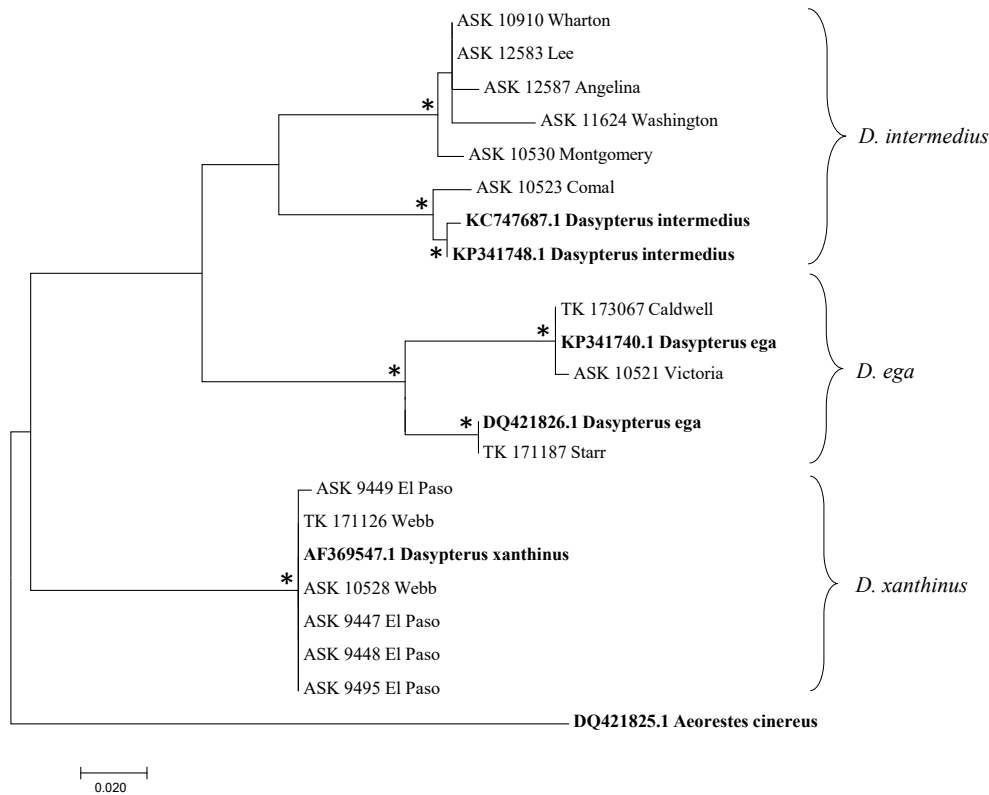


FIGURE 1. Neighbor-joining tree generated from 258 base-pair fragments of the cytochrome *b* gene from yellow bat specimens recovered from the Texas Department of State Health Services. Sample names are based on collection tissue numbers and county of origin. Reference sequences from Genbank are in bold and were included to identify clusters. The Hoary Bat (*Aeorestes cinereus*) was used as an outgroup. An asterisk (*) at nodes indicates high bootstrap support (> 90%).

System (Beckman Coulter Inc., Brea, California). We sent samples for Sanger sequencing, amplified using the LGL765/LGL766 primer set, to the Genomic Core Sequencing Lab at Texas A&M, Corpus Christi, Texas.

All sequences were edited and compared to reference sequences of the same species with Sequencher v. 5.1 (Genecodes Corp., Ann Arbor, Michigan). Consensus sequence data were then exported and aligned in MEGA 7 (Kumar et al. 2016) using the MUSCLE alignment tool. We analyzed aligned sequences and we constructed a neighbor-joining tree using a 258 base-pair fragment of *Cytb* to show the phylogenetic relationships between the *Dasypterus* species (Fig. 1). Nodal support was evaluated with 1,000 bootstrap replicates. We included reference sequences of known species of yellow bats from Genbank (GB) in the alignment (*D. ega*: DQ421826 and KP341740.1; *D. intermedius*: KC747687.1 and KP341748.1; *D. xanthinus*: AF369547.1). We also included a sequence from a Hoary Bat (*Aeorestes cinereus*) from GB (DQ421825.1) to be used as an outgroup. We confirmed specimen identification by the clustering pattern and by the similarity to reference *Cytb* sequences. Once we confirmed species identities, we created updated distribution maps with QGIS 3.4 (QGIS Development Team 2019) indicating new county records or those updated from Texas DSHS records.

RESULTS

***Dasypterus ega* (Southern Yellow Bats).**—Southern Yellow Bats in the U.S. are known only from the southernmost counties of Texas. Here, we present eight new county records for Southern Yellow Bats (Fig. 2A) and extend the northern limit of their range into Travis and Montgomery counties in Texas. A specimen from San Patricio County represents an update from a Texas DSHS record to a collections-based specimen record.

Bandera County.—A male specimen (ASNHC17404, ASK11627) was received on 12 February 2014.

Caldwell County.—A male specimen (TTU114567, TK173067) was received on 9 June 2011. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB accession MN895072).

Hays County.—Two specimens were recovered from Hays County. A male specimen (ASNHC17403, ASK11626) was collected on 5 March 2014. A female specimen (ASNHC19953, ASK13748) was collected on 22 January 2018.

Montgomery County.—A specimen of undetermined sex (ASNHC16290) was received on 8 February 2013.

San Patricio County.—A female specimen (ASNHC18197, ASK12697) was collected on 24 October 2013.

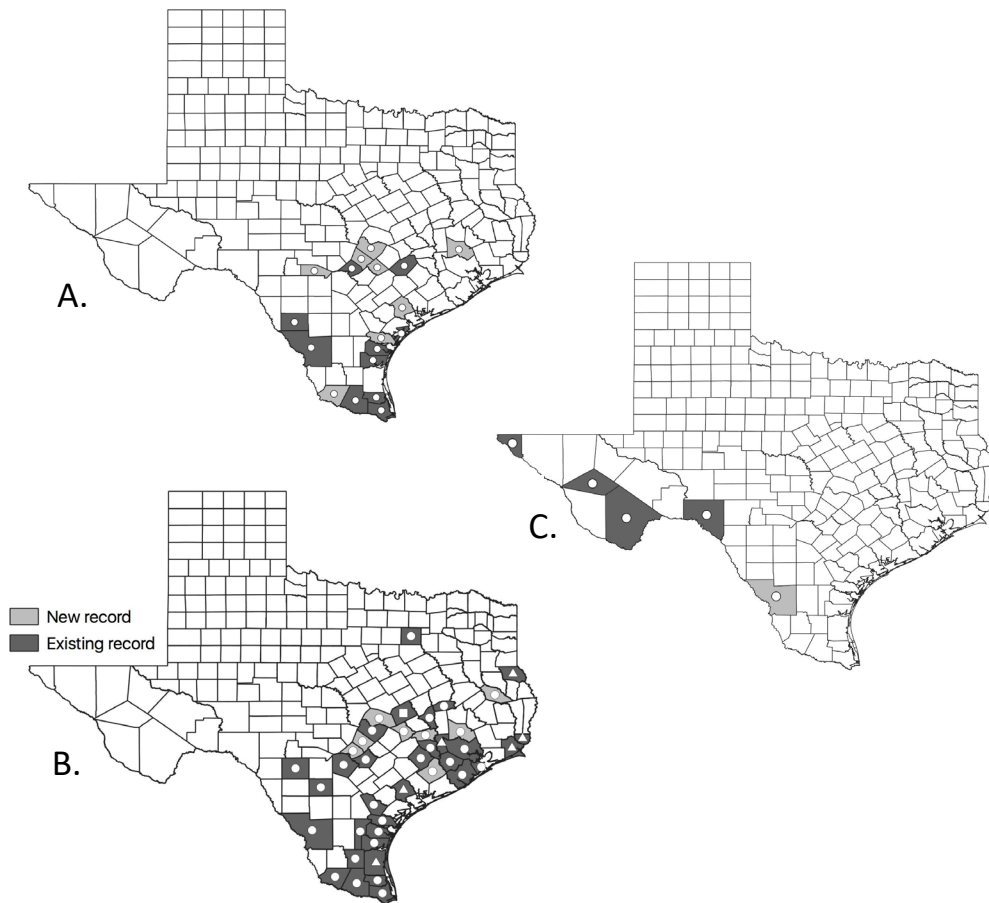


FIGURE 2. Updated distribution maps for (A) Southern Yellow Bats (*Dasypterus ega*), (B) Northern Yellow Bats (*D. intermedius*), and (C) Western Yellow Bats (*D. xanthinus*). New county records are shaded with light gray. Symbols are circles = specimen vouchers, squares = literature records, triangles = Texas Department of State Health Services records.

Starr County.—A female specimen (TTU113575, TK171187) was received on 11 July 2008. We confirmed the species identity (Fig. 1) with *Cytb* (GB MN895073).

Travis County.—A male specimen (ASNHC19335, ASK13746) was collected on 5 September 2018.

Victoria County.—A male specimen (ASNHC15835, ASK10521) was collected on 29 June 2012. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MN895074).

***Dasypterus intermedius* (Northern Yellow Bats).—**

Northern Yellow Bats have been primarily collected in counties in Texas along the coast of the Gulf of Mexico, but recently appear to be expanding inland (Demere et al. 2012). We present eight new county records for Northern Yellow Bats (Fig. 2B), including new collections-based specimen records for two counties that had previously been considered Texas DSHS records (Montgomery and Wharton counties). These records are from counties within the known distribution of Northern Yellow Bats.

Angelina County.—A female specimen (ASNHC17877, ASK12587) was collected on 7 September 2016.

We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MK876232).

Comal County.—A male specimen (ASNHC15836, ASK10523) was received on 11 September 2012. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MN895076).

Hays County.—A female specimen (ASNHC19952, ASK13747) was received on 17 October 2018.

Lee County.—A male specimen (ASNHC17873, ASK12583) was collected on 6 October 2016. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MK876234).

Montgomery County.—Two specimens were recovered from Montgomery County. A male specimen (ASNHC15863, ASK10530) was received on 17 July 2012. We confirmed the species identity this specimen (Fig. 1) with *Cytb* (GB MK876235). An additional male specimen (ASNHC17872, ASK12582) was collected on 15 September 2016.

Washington County.—A male specimen (ASNHC17401, ASK11624) was received on 7 March 2014. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MK876252).

Wharton County.—A male specimen (ASNHC19059,

ASK10910) was received on 23 June 2015. We confirmed the species identity of this specimen (Fig. 1) with *Cytb* (GB MK876253).

Williamson County.—A male specimen (ASNHC19300, ASK13849) was received on 13 October 2014.

***Dasypterus xanthinus* (Western Yellow Bats).—**

Since Western Yellow Bats were first documented in Texas, the species has been primarily reported in the Big Bend and Trans-Pecos regions and has only been collected in Texas in Brewster, Jeff Davis, Val Verde, and El Paso counties. Here, we report an additional county in which Western Yellow Bats have been collected (Fig. 2C). Specimens from Webb County represent the easternmost records of Western Yellow Bats in the U.S. This eastern extension increases the difficulty of identifying yellow bats to species level as the three species have all been reported in Webb County and their ranges likely overlap in other counties in southern Texas.

El Paso County.—Western Yellow Bats have been previously reported in El Paso County (Tipps et al. 2011); however, those specimens tested positive for rabies virus and may have exhibited abnormal behavior. Here, we report four additional records of Western Yellow Bats collected in El Paso County, all of which tested negative for rabies virus to corroborate previous records. A female specimen (ASNHC15826, ASK9495) was received on 30 July 2012. An additional female specimen (ASNHC16080, ASK9449) was collected on 04 September 2012. A male specimen (ASNHC16079, ASK9448) was collected on 21 September 2012. An additional male specimen (ASNHC16078, ASK 9447) was received on 26 September 2012. We confirmed the species identity for these four specimens (Fig. 1) with *Cytb* (GB MN895077, MN895078, MN895079, MN895080, respectively).

Webb County.—Two specimens were collected from Webb County. A female specimen (TTU113514, TK171126) was collected on 25 June 2009. We confirmed the species identity for this specimen with *Cytb* (GB MN895081). Additionally, a male specimen (ASNHC15800, ASK10528) was collected on 19 July 2012. We confirmed the identity of this specimen (Fig. 1) with *Cytb* (GB MN895082).

DISCUSSION

Specimens submitted to state health departments offer unique insight into the distributions of elusive species, especially in areas that are not sampled frequently with conventional collection methods (Yancy and Jones 1996; Tipps et al. 2011; Demere et al. 2012). Documenting shifts in species distributions with new county records provides valuable data to help monitor the status of populations and diseases, and gives insight into life-

history traits such as habitat preferences and migration habits. It is also important to document that such changes as anthropogenic activity and climate change alter habitat suitability for many species. Though the three species of yellow bats that occur in the U.S. are listed as species of least concern on the Red List of the International Union for Conservation of Nature (IUCN), Southern Yellow Bats are listed as threatened in Texas (Texas Parks and Wildlife. 2019. Federal and State Listed Mammals in Texas. Available from <https://tpwd.texas.gov> [Accessed 22 November 2019]) and Western Yellow Bats are listed as threatened in New Mexico (Jones and Schmitt 1997).

The possible range expansion of yellow bats has been hypothesized to correspond to the introduction of ornamental palms in residential areas, as noted in Western Yellow Bats in southern California (Constantine 1998). Though the use of ornamental palms in landscaping can provide more roosting sites for yellow bats, the practice of pruning the dried leaves puts roosting bats at risk of losing roosts or getting injured when the leaves are removed (Mirowsky 1997; Zabriskie et al. 2019). Other major threats that affect these species are pesticide use (Clark et al. 1978; Clark 2001) and collisions with wind turbines (Arnett et al. 2016). As anthropogenic activity continues to alter landscapes and change habitat suitability for other organisms, documenting range shifts and monitoring population trends will be necessary for effective conservation assessment of bat species.

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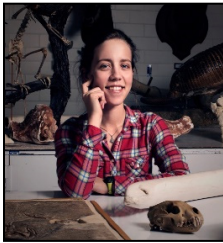
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SYDNEY K. DECKER is currently a Ph.D. student at The Ohio State University, Columbus, Ohio, in the Department of Evolution, Ecology, and Organismal Biology. She completed her Bachelor of Science in Biology at Angelo State University, Texas. Sydney is interested in using genomics and modeling to look at phylogeography and speciation in bats. (Photographed by Jamie Huff).



DIANNA M. KREJSA is the Collections Manager of the five divisions of the Angelo State Natural History Collections (ASNHC) at Angelo State University (ASU) in San Angelo, Texas. She received her Bachelor's in Biology from Simpson College, Indianola, Iowa, and a Master of Science degree from the University of New Mexico, Albuquerque, where she worked extensively within the Museum of Southwestern Biology. She has been with ASU since 2017. (Photographed by Angelo State University Department of Communications and Marketing).



LARAMIE L. LINDSEY is a Post-doctoral Researcher in the Veterinary and Biomedical Sciences Department at the University of Minnesota, Minneapolis. Laramie completed her Bachelor of Science at Southern Methodist University, Dallas, Texas, and her Master of Science at Angelo State University, Texas, before completing her Ph.D. at Texas Tech University, Lubbock. Currently, she is using next-generation sequencing to construct viromes of rodents at the rodent-Ag interface. (Photographed by Gage Rowden).



RICHARD P. AMOATENG is a current intern doctor at Connolly Hospital, Blanchardstown, Dublin, Ireland. He is a Postgraduate Year 1 and completed his medical degree at University College Cork School of Medicine, Ireland, May 2019. He holds a Bachelor of Science in biology with a minor in chemistry from Angelo State University, Texas. Richard will start his internal medicine residency training program in the U.S. in July 2020. (Photographed by Kwame Amoateng).



LOREN K. AMMERMAN is the C. J. Davidson Endowed Professor of Biology at Angelo State University (ASU) in San Angelo, Texas, and serves as the Curator of Genomic Resources in the Angelo State Natural History Collection. She has been with ASU since 2001 and has mentored 29 graduate student thesis projects, advised 25 undergraduate research efforts, published 51 articles in peer-reviewed journals with students and colleagues, and received 23 research grants. Her projects with students range from documenting distributions of species to life-history investigations (such as diet analyses, roosting ecology) to population genetics and molecular systematics of mammal species. (Photographed by Angelo State University Department of Communications and Marketing).