MORPHOLOGICAL AND MOLECULAR VARIATION IN TOWNSEND'S BIG-EARED BAT (*CORYNORHINUS TOWNSENDII)* IN WEST TEXAS

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ABSTRACT

Previous studies of Townsend's big-eared bat (*Corynorhinus townsendii*) from Big Bend National Park (Brewster Co.) have been limited and inconclusive regarding the expected subspecific identity of specimens from this region. Furthermore, we observed that several specimens of from this region displayed morphological characteristics of both the Mexican big-eared bat (*C. mexicanus*) and *C. townsendii*. Thus, the goals of this study were to use molecular data to determine the specific and subspecific affinity of the specimens found in this region and to illuminate possible morphological variation within the molecular lineages recovered in West Texas specimens. Based on molecular analyses there was support for the presence of a single subspecies, *C. t. australis*, throughout West Texas. Evaluation of morphological data from these same specimens showed that no discrete characteristics were accurate in delineating *C. townsendii* from *C. mexicanus*, confirming substantial morphological variation within *C. t. australis* specimens from West Texas.

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INTRODUCTION

Two species of vespertilionid bats in the genus *Corynorhinus* occur in the southern United States and Mexico (Ceballos and Oliva 2005; Hall 1981; Handley 1959; Kunz and Martin 1982; Piaggio and Perkins 2005; Tumlison 1991; Tumlison 1992). Townsend's big-eared bat (*C. townsendii)* can be found throughout much of western North America extending into southern Mexico, with two subspecies (*C. t. ingens* and *C. t. virginianus*) isolated in the eastern United States (Fig. 1). The Mexican big-eared bat (*C. mexicanus*) is endemic to Mexico and can be found in the higher elevation of the Sierra Madre Occidental, Sierra Madre Oriental, and the Trans-Volcanic Belt. In areas of sympatry in Mexico, the two species (*C. townsendii* and *C. mexicanus*) have been found to cohabitate in caves or mine shafts (López-González and Torres-Morales 2004). Previously, there was no reason to question the identity of specimens captured in the Chihuahuan desert ecoregion of Texas; however, recent work in Big Bend National Park (Brewster Co., Tx) found *C. townsendii* specimens with a second cusp on the upper first incisor, a trait normally only found in *C. mexicanus* or *C. townsendii ingens* (Handley 1959; Kunz and Martin 1982). Although *C. townsendii* is not expected to display this character, Handley (1959) noted that this species is one of the most variable in terms of morphology. He also stated that although *C. townsendii* usually lacks a second cusp on the first incisor, it is variable among the subspecies because it is found in *C. t. ingens*.

Handley (1959) and Jones (1977) defined five distinct morphological characteristics that distinguish *C. townsendii* from *C. mexicanus*: length of the skull, length of the maxillary toothrow, tragus length, number of interfemoral cross ribs, and the number of cusps on the

first incisor (Table 1). As we investigated the occurrence of this accessory cusp on the incisor in bats of West Texas, we noticed that several of these key characteristics were more variable than previously thought. For example, one of the specimens we examined had the tragus length, length of skull, and incisor of *C. townsendii* but had the number of interfemoral cross ribs and length of the maxillary toothrow characteristic of *C. mexicanus*. Several of the specimens we examined from Brewster Co. had a mixture of characters such as this, leading us to question their identity.

Not only was the species in this area in question, but the subspecies was as well. Five subspecies are currently recognized within *C. townsendii*: *C. t. ingens*, *C. t. virginanus*, *C. t. townsendii*, *C. t. australis*, and *C. t. pallescens*. Subspecies are described as being geographic races of a certain species (Mayr 1963). Although they are geographically distributed, these populations are not reproductively isolated. Until populations show significant adaptive differentiation or genetic divergence, a sign that the subspecies is on its own path to speciation, they are still considered geographic races of a single species (e.g. Wan et al. 2005). Bats of the genus *Corynorhinus* are a good example of these geographically distributed subspecies because bats in this genus tend to have low vagility and are highly philopatric (Humphrey and Kunz 1976, Piaggio and Perkins 2005, Weyandt et al. 2005). The five subspecies of *C. townsendii* were delineated based on different morphological features present over the large geographical range of the species as described by Handley (1959). This particular method of subspecies delineation has been used in several different species of bats (*Centurio senex*-Paradiso 1967; *Eptesicus fuscus*-Hoffman and Genoways 2008; *Eumops floridanus*-Timm and Genoways 2004). Additionally, molecular

divergence has become a preferred method to assess species and subspecies limits (Evin et al. 2008; Lausen et al. 2008; Piaggio and Perkins 2005; Rossiter et al. 2007). Recently, studies have taken a combined approach, including both morphological and molecular data, to aid in the delineation of both species and subspecies (Baker et al. 2009; Cardinal and Christidis 2000; Larsen et al. 2010; Lee et al. 2010; McDonough et al. 2008; Rodriguez and Ammerman 2004).

Originally, Handley (1959) suggested that *C. t. australis* was the subspecies found in West Texas based on morphological data; however, he also suggested a zone of intergradation in the Chihuahuan Desert region of Texas between *C. t. australis* and *C. t. pallescens.* Handley (1959) described *C. t. australis* as having a darker coloration and slightly larger measurements (based on greatest skull length, zygomatic arch, breadth across the orbitals, length of maxillary tooth row, depth of brain case, postpalatal length, and breadth across the palate). In comparison, *C. t. pallescens* was described as having the lightest pelage (almost yellow in color) and was slightly smaller in size. Nevertheless, compared to the other three subspecies, these two subspecies are medium in size. Additionally, Handley (1959) described some specimens from Big Bend National Park to be lighter in color, thus being more like *C. t. pallescens*, whereas others were described as slightly darker in color, looking more like *C. t. australis.* In comparison with *C. townsendii*, *C. mexicanus* has a darker pelage (Tumlison 1991). Furthermore, neither of the two subspecies (*C. t. australis* and *C. t. pallescens*) is expected to display a bilobed incisor. Therefore, species misidentifications between *C. mexicanus* and *C. townsendii* are less likely to occur in areas of sympatry.

In order to further investigate the genus *Corynorhinus* in Mexico, Tumlison (1991) conducted a morphometric analysis on *C. t. pallescens*, *C. t. australis*, and *C. mexicanus*, mainly in northern Mexico but also throughout their ranges. Tumlison (1991) found that *C. townsendii* was the largest species and that C. *t. australis* was the largest of the three taxa overall. However, he found difficulty in distinguishing specimens found in northern Mexico as either *C. t. australis* or *C. t. pallescens*, which supports Handley's (1959) proposal that a zone of intergradation exists between the two subspecies. Additionally, Smith and Tumlison (2004) investigated populations of *C. townsendii* located along the Texas-Oklahoma border and the Kansas-Oklahoma border using the same methods used by Tumlison (1991). The results of their study illustrated clinal variation with larger specimens to the north and smaller specimens to the south. Thus the authors concluded that differences in geography played a role in the morphological variation of these two subspecies.

Molecular studies have been conducted at both deep and shallow taxonomic levels for the genus *Corynorhinus* (generic level: Bogdanowicz et al. 1998; Frost and Timm 1992; Hoofer and Van Den Bussche 2001; species level: Piaggio and Perkins 2005; subspecies level: Piaggio et al. 2009; Weyandt et al. 2005). Piaggio and Perkins (2005) conducted an extensive study to test the phylogenetic relationships within the genus *Corynorhinus.* Results of that study supported the designation of the five subspecies of *C. townsendii* in North America and Mexico, but modified their geographic distributions (Fig. 1). Piaggio and Perkins (2005) suggested the distribution of *C. t. pallescens* was limited to New Mexico, Colorado, and small areas in Texas and Oklahoma, instead of a wider distribution spanning the western half of the United States extending into Canada as suggested by Handley (1959).

Conversely, the range of *C. t. australis* stayed the same, supporting Handley's (1959) previous description that this subspecies was only found in Texas, the southern tip of New Mexico, and throughout Mexico (Fig. 1). However, Piaggio and Perkins (2005) analyzed only two specimens of *C. townsendii* from Presidio Co. in Texas with sequence data from one mitochondrial gene (control region) and concluded that *C. t. australis* was the subspecies found in this region. With such limited data, the application of these results to all western Texas specimens is questionable.

The main objectives of this study were to use both morphological and molecular data to determine the specific and subspecific affinity of *Corynorhinus* specimens from West Texas and to elucidate the extent to which the characters defined by Handley (1959) and Jones (1977) vary in this population of *Corynorhinus* in the Chihuahuan Desert ecoregion of Texas. We hypothesized that these specimens were the species *C. townsendii,* more specifically the subspecies *C. t. australis.* We also hypothesized that there was more morphological variation in *C. townsendii* than previously thought.

MATERIALS AND METHODS

Taxonomic Sampling.— A total of 92 *Corynorhinus townsendii* specimens were used in this study (Appendix I) from six counties in the Chihuahuan Desert ecoregion of Texas (Brewster Co., Culberson Co., Hudspeth Co., Jeff Davis Co., Presidio Co., and Val Verde Co.) (Fig. 2). Specimens were acquired from the Angelo State Natural History Collections (Angelo State University), Natural Science Research Laboratory (Texas Tech University) and the Museum of Southwestern Biology (University of New Mexico). For the morphological analysis, 68 specimens were measured and used for data analysis. For the molecular analysis, 59 specimens were sequenced, which included 22 wing punches collected in Big Bend National Park in July 2010. Finally, 34 specimens were used in both the molecular and morphological analysis.

An additional 20 sequences including four out of the five subspecies of *C. townsendii*, other species in the genus *Corynorhinus, C. mexicanus* and *C. rafinesquii*, and two *Barbastella* sequences were downloaded from GenBank (www.ncbi.com; Appendix II). *C. rafinesquii* was used as an outgroup because it is within the same genus and sister to the *C. townsendii-C. mexicanus* clade (Piaggio and Perkins 2005). Additionally, *Barbastella* was used as an outgroup outside of the genus *Corynorhinus* (Lack and Van Den Bussche 2010). A single *Corynorhinus mexicanus* specimen was used for group comparison in the morphological analysis.

Molecular Techniques.— Whole genomic DNA was extracted from either frozen liver tissues or wing punches stored in 95% alcohol. DNA isolation was conducted using a DNeasy Tissue Kit (QIAGEN, Inc., Valencia, California) following the manufacturer's protocol for tissue extraction. Successful extraction of the DNA was confirmed by using a

0.8% agarose gel for visualization. DNA was quantified using a Qubit fluorometer and the Quant-iT DNA Assay Kit, Broad Range (Invitrogen, Grand Island, New York).

The mitochondrial gene, cytochrome *b*, was then amplified using polymerase chain reaction (PCR). Previously published primer sequences for cytochrome *b* (GLUDG-L-Palumbi et al. 1991; HCB-Piaggio and Perkins 2005) designed to work for *Corynorhinus* and other vertebrates were used in the PCR reaction. PCR reactions totaled 12.5 µl and consisted of the following reagents: 1x reaction buffer, 0.16 µM of each forward and reverse primer, 2 mM $MgCl_2$, 0.16 mM deoxynucleotide mix, and 1U Taq polymerase (5 units/µL; New England BioLabs, Ipswich, Massachusetts). Each reaction contained approximately 50-200 ng of DNA template. The reaction was amplified using a thermal cycler (MyCycler, BioRad, Hercules, California) and the thermal profile for these reactions began with an initial denaturation of 92°C for 2 min, followed by 39 cycles of 94°C for 1 min, annealing for 1 min at 48°C and 72°C for 1 min, with a final extension of 72°C for 10 min. Once samples were amplified, PCR products were visualized using a 0.8% agarose gel.

Good quality PCR products were quantified using a Qubit fluorometer and the Quant-iT DNA Assay Kit, High Sensitivity. The samples were then purified using Exosap-IT (Affymetrix, Santa Clara, California). Samples were prepared for sequencing using the GenomeLab-DTCS Quick Start Kit and sequenced on the Beckman-Coulter CEQ8000 genetic analyzer (Beckman-Coulter Inc., Fullerton, California). We followed the manufacturer's protocol for sequencing but used quarter reactions instead of the full reaction amount suggested by the manufacturer. Overlapping sequences were aligned using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan) and ambiguities were

resolved by eye. Sequences downloaded from Genbank (Appendix I) were added to the alignment. Coding regions were translated to act as a second check for sequence alignment.

The final alignment was exported to MEGA5 (Tamura et al. 2011). We used the likelihood analysis in MEGA5 to select the best model of evolution for this dataset based on Bayesian Information Criterion (BIC) and Aikake's Information Criterion (AIC). We then used a Maximum Likelihood (ML) analysis in MEGA5 to create a phylogenetic tree using the best fit model of evolution. Bootstrap analyses (1000 pseudoreplicates) using ML criteria were used to test branch significance and bootstrap values >70 (Hillis and Bull 1993) were considered significant. Average genetic distances were calculated using the HKY + G model of evolution in MEGA5.

Bayesian methods also were used to test the phylogenetic relationships among the samples in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Two simultaneous runs of 10 million generations and the model HKY + G was used in this analysis. Convergence of the two runs was considered when the average standard deviation of split frequencies was less than 0.01. Trees were sampled every 100 generations and a burn-in to discard the first 25% of saved trees was used. Nodes with Bayesian posterior probability values >0.95 were considered significant nodes on the Bayesian tree (Huelsenbeck and Ronquist 2001).

Morphological Techniques.—The total length of skull (TL), breadth across zygomatic arch (ZB), breadth across cranium (CB), breadth across mastoid (MB), post-orbital constriction (POC), maxillary tooth row (MT), palatal length (PL), basiocranial length (BL), auditory bullae length (ABL), intercanine width (ICW), palatal breadth across 3rd molar

(PBM3), interpterygoid width (IPW), cranial depth (CD), dentary length (DL), moment arm of temporal (MAT), moment arm of mandible (MAM), coronoid to angle distance (CA), and mandibular fossa to condyle width (FC) of the skull were measured three times using digital calipers to the closest 0.01 mm when intact on the study skull (Tumlison 1991; Table 2). We then averaged these values for use in later analyses. Additionally, we counted the number of interfemoral cross ribs. Standard measurements including measurements of the tragus and ear were recorded from data tags when associated with a study skin. We scored the first incisor of *C. townsendii* and *C. mexicanus* as one of three character states (1-Presence of a second cusp on both upper incisors, 2-presence of a second cusp on one upper incisor, 3-absence of a second cusp on both upper incisors).

All analyses were conducted using R statistical computing language (R Development Core Team 2012). We then constructed a data matrix based on the measurements taken from each skull and a created a Bray-Curtis dissimilarity matrix (Bray and Curtis 1957). We analyzed two different data sets; one with only the 18 skull measurements and one with the skull measurements with the addition of 2 characteristics- the accessory cusp on the incisor and number of interfemoral crossbars. Data were analyzed using cluster analysis in order to determine if there was any natural grouping among the samples (Romesburg 1984). The unweighted pair-group with arithmetic mean algorithm was used to create a dendrogram showing these natural groupings. The number of groups in the cluster analysis was set *a priori* to two because we expected only two taxa to be present in this analysis. Finally, we tested the significance of these groups by running an analysis of similarities (ANOSIM) test. ANOSIM is a non-parametric test used to determine whether groups are significantly

different between groups or within groups. With ANOSIM, an R value is computed by comparing between group distances and within group distances. Thus, R values closest to one show dissimilarity between groups, while R values close to zero are considered to be similar groups (Clarke 1993). Significance of the R value was then tested through 1000 permutations of group membership.

Finally, diagnostic characteristics in the literature (Table 1; Handley 1959, Jones 1977) that distinguish *Corynorhinus townsendii* from *Corynorhinus mexicanus* were also evaluated to determine which characteristics were most useful in differentiating between the two species. This was accomplished by calculating the percentage of specimens from West Texas with *C. townsendii*-like characteristics and comparing those percentages to specimens from West Texas with *C. mexicanus*-like characteristics.

RESULTS

Molecular Analysis.—We were able to successfully sequence and align 951 bases of the cytochrome-*b* gene from 59 *Corynorhinus townsendii* individuals. A ML tree was obtained (-ln=3826.79) using the HKY+G model of evolution (Fig. 3) using the following parameters: base frequencies=0.278, 0.261, 0.306, 0.156; 2 substitution types; and gamma shape parameter = 0.34. After 10 million generations in the Bayesian analysis, the average standard deviation of split frequencies was 0.00965. Topology of the two trees was similar with only minor differences in the groupings of *C. t. australis* specimens (Fig. 3). Significant nodes in the ML tree were significant in the Bayesian tree as well. Both trees supported a *C. mexicanus* clade, in which none of the specimens from Texas were found. Additionally, within the *C. townsendii* clade, we found support for the separation of a *C. t. australis*-*C. t. pallescens* clade from the other subspecies in *C. townsendii*. Finally, both trees significantly supported a *C. t. pallescens* clade that did not include any samples from West Texas (ASK8698 is a specimen from Hutchinson Co. in the Texas panhandle).

Divergence (HKY+G) within *C. t. australis* was between 0% and 1.9%, while divergence between *C. t. australis* and *C. t. pallescens* ranged from 0.8% to 2.3%. Finally, divergence between *C. townsendii* and *C. mexicanus* ranged from 6.3% to 8.1%.

Morphological Analysis.—Averages of each measurement were within the expected range for *C. townsendii* for both males and females (Table 2). Tumlison (1991) showed that there is significant sexual dimorphism between males and females within *C. townsendii*; however, this was taken into account in the cluster analysis. The cluster analyses delineated our specimens into two groupings in the resulting dendrogram (Fig. 4). Both data sets, one with incisor and interfemoral crossbar data and the one without these data points, reported

the same group membership. The first group consisted of *C. mexicanus* (TTU 56970) and a single *C. townsendii* specimen (ASNHC 14316; ASK 8684), while all other specimens used for the morphological analysis grouped together in a *C. townsendii* cluster. There was no evidence to support structure within the *C. townsendii* group, but no *C. t. pallescens* specimens were included in the analysis. The ANOSIM test produced an R value equal to 0.9422, showing that these two groups are significantly different ($p=.001$). The single *C. townsendii* specimen that clustered with *C. mexicanus*, ASNHC 14316 (Brewster Co., Texas), showed 4 of the characteristics that distinguish *C. mexicanus* from *C. townsendii*.

Additionally, we investigated the occurrence of *C. mexicanus*-like characteristics in *C. townsendii* specimens from West Texas. We found that none of these presumably diagnostic characteristics were completely accurate for identifying the species (Table 3). The characteristic that was most reliable was the presence or absence of the secondary cusp on the first incisor. This characteristic only misidentified the specimens 19% of the time. Conversely, the length of the maxillary toothrow was the least reliable in differentiating between the two species, misidentifying the specimens 39% of the time. Notably, 19 of the specimens with *C. mexicanus*-like characteristics shared at least two or more of the *C. mexicanus*-like characters with some sharing 4 out of the 5 distinguishing characters. Finally, 11 of these 19 specimens were included in the molecular analysis and had *C. townsendii* haplotypes.

DISCUSSION

Molecular Analysis.— Overall, our gene tree based on cytochrome *b* data supported the hypothesis that *Corynorhinus townsendii* is the species found in West Texas, more specifically it is the subspecies *C. t. australis*. These molecular data recovered the same relationships among the different taxa within the genus *Corynorhinus* as those presented in Piaggio and Perkins (2005). We did not find any evidence to show that *C. mexicanus* has expanded its range into West Texas. Additionally, divergences found between *C. townsendii* and *C. mexicanus* specimens were consistent with the levels of sequence difference seen between sister taxa (Baker and Bradley 2006) and those found between *C. townsendii* and *C. mexicanus* in Piaggio and Perkins's (2005) study.

Furthermore, molecular data also confirmed *C. t. australis* as the subspecies found in West Texas. Theoretically, if *C. t. australis* and *C. t. pallescens* lived in an area of sympatry, the two would still interbreed in a zone of intergradation and we would expect to see evidence of both subspecies in the cytochrome *b* gene tree. Hoffman and Genoways (2008) characterized one such zone found between two *Eptesicus fuscus* subspecies in Nebraska, concluding that there was a zone of intergradation running northeast to southwest in the state. Thus, gene flow between the two populations was still occurring. However, because we did not find any genetic evidence of *C. t. pallescens* in this area, this does not seem to be the case with *C. townsendii* subspecies in West Texas. Thus, Handley's (1959) hypothesis that a zone of intergradation occurs between the two subspecies of *C. townsendii* was rejected.

Additionally, although not included in the final molecular analysis, we were able to successfully obtain approximately 50 bases of sequence data from museum specimens from northern West Texas in Culberson Co. (TTU 19960 and TTU 23277). When these sequences

were compared in the full alignment, they clustered with *C. t. australis*. These data suggest that the range of *C. t. australis* extends farther north than previously thought with no intergradation between *C. t. pallescens* and *C. t. australis* (Handley 1959; Piaggio and Perkins 2005; Tumlison 1991). However, we would need more samples from New Mexico, North Texas, and Mexico to test the limits of the distribution of *C. t. australis* and *C. t. pallescens.*

Morphological Analysis.—What Handley (1959) deemed a zone of morphological intergradation in Texas and Northern Mexico may actually be clinal variation in morphology of *C. townsendii* specimens from north to south. We saw a decrease in the averages for the different skull measurements as we moved southward (Table 3), which supports this idea of clinal variation in *C. townsendii* specimens in West Texas and agrees with Bergmann's rule, which states that larger organisms are found in colder climates while the smaller sized organisms will be found in warmer climates (Bergmann 1847 translated in James 1970). Several other studies describe similar clinal variation in the morphology of various bat species (*Myotis daubentonii,* Bogdanowicz 1990; *Cynopterus sphinx,* Storz et. al 2000; *Eptesicus fuscus*, Hoffman and Genoways 2008).

Based on the cluster analysis of morphological characteristics (Fig. 4), we saw a significant ($p=0.001$) difference between the two species, with *C. townsendii* and *C. mexicanus* clustering in their respective groups. Although not all of the specimens in the morphological analysis were included in the molecular study, the 34 that overlapped between the two studies were all found to be *C. townsendii* based on both morphology and molecular data. It should be noted that the single *C. townsendii* specimen, ASNHC 14316, that clustered

with *C. mexicanus* was also tested using molecular data. Based on these genetic data, ASNHC 14316 is *C. townsendii*; however, overall ASNHC 14316 had smaller measurements than any of the other *C. townsendii* specimens and shared 4 out of the 5 *C. mexicanus*-like characters, which could be why it clustered with the *C. mexicanus* specimen.

Morphological Variation in C. townsendii.— Because of the high morphological variation seen in individuals that clustered as *C. townsendii* in the molecular analysis, we can conclude that there is more morphological variation within *C. townsendii* than previously thought. ASNHC 14316 from Big Bend National Park, Brewster, Co. is a good example to show this increased morphological variation in *C. townsendii*. This specimen was included in both the molecular and morphological analyses and shared 4 of the 5 diagnostic characteristics that differentiate *C. mexicanus* (Table 1). Genetically, ASNHC 14316 is *C. townsendii*. The fact that this specimen groups with the single *C. mexicanus* specimen shows that characteristics that have been used to differentiate between the two species are unreliable in West Texas. The unreliability of these presumed key characteristics in West Texas, especially the length of the maxillary toothrow, lead us to conclude that a more stringent test of species identification is needed for this area. Especially in areas of sympatry, such as in Northern Mexico, species determination should be confirmed using some type of molecular data, such as cytochrome *b* sequences.

Although we did find that the accessory cusp was the most reliable characteristic to identify *C. townsendii* or *C. mexicanus*; it is interesting to note that two of the specimens we examined in the morphological analysis in this study had only one of the two upper incisors with an accessory cusp. Therefore, not only did we see variability in specimens with the

presence or absence of an accessory cusp on the incisors, but we also saw variability in the number of upper incisors with the accessory cusp.

Variation in the number of accessory cusps on a tooth, specifically the incisor, has been documented by Velazco et al. (2010) in several species of *Platyrrhinus*. Salazar-Ciudad and Jernvall (2002) evaluated tooth cusp formation during development and expressed that it is controlled either by an activator or an inhibitor on a tooth cusp gene that creates or prevents the creation of cusps on the tooth. They suggested that minor changes within a gene sequence can cause large morphological shifts and that similar morphologies could be caused by different genetic changes. Thus, the basis of an accessory cusp on the upper incisor in *C. townsendii* could be caused by a single base mutation in one of these cusp-growth activator genes.

Possible C. townsendii-C.mexicanus *hybridization.*—A single mitochondrial gene was used to test the relationships between these specimens; therefore, we can say with certainty that the maternal lineage of our specimens from West Texas was *C. townsendii*. However, this does not rule out the possibility of hybridization between *C. townsendii* and *C. mexicanus*. Because we did not detect any *C. mexicanus* haplotypes in West Texas, hybridization between the two species would have to be between male *C. mexicanus* and female *C. townsendii*.

Mao et al. (2010) used molecular data to test for the presence of male-mediated introgression in both species and subspecies of horseshoe bats. They found that the topologies of their mitochondrial gene trees were vastly different than those of their nuclear gene trees. Thus, they concluded that only looking at mitochondrial genes is not enough to

completely understand the genetic histories of these bat species. In order to test the hypothesis that hybridization is occurring, we would need to include several other nuclear markers, such as microsatellites or AFLP. Multiple genetic data sets are crucial in gaining a full understanding of the genetic histories of bat species (Petit and Excoffier 2009). Additionally, more samples from other counties in New Mexico and West Texas, more specifically El Paso Co., Terrell Co., and Hudspeth Co., would help us understand the degree of gene flow between populations in this area and to determine how the variation in the accessory cusp on the incisor is distributed.

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Fig. 1 — Distribution map for *Corynorhinus townsendii* subspecies compiled from descriptions of distributions found in Handley (1959), Tumlison (1991), Smith and Tumlison (2004), Piaggio and Perkins (2005), and Smith et al. (2008).

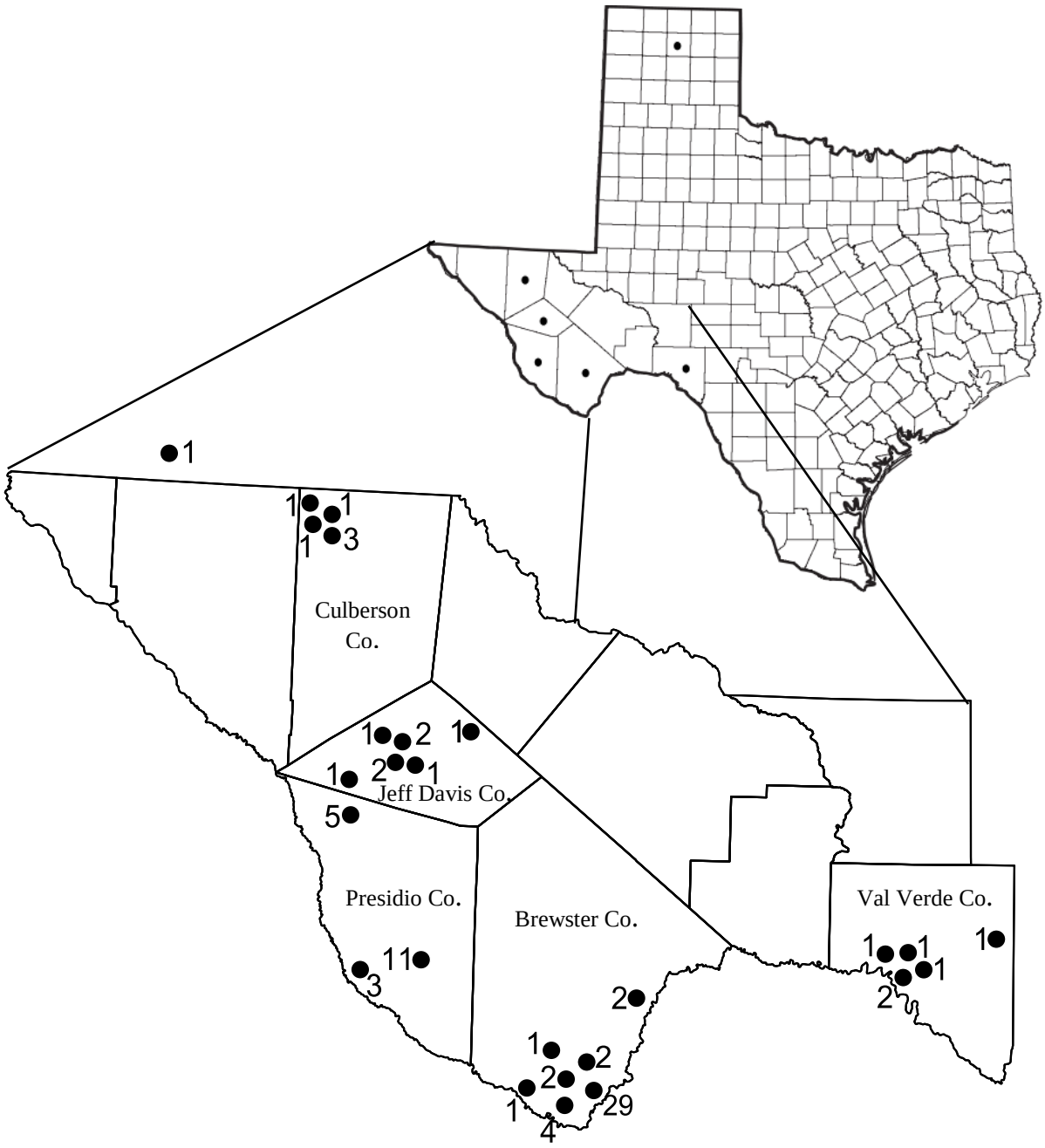

Fig. 2 — Sites where *Corynorhinus townsendii* were collected in Texas and New Mexico. The number next to the location is the number of specimens used from that particular site. A single *C. t. pallescens* specimen was from Hutchinson Co. in the Texas Panhandle. Specimens collected from Mexico are not shown on this map.

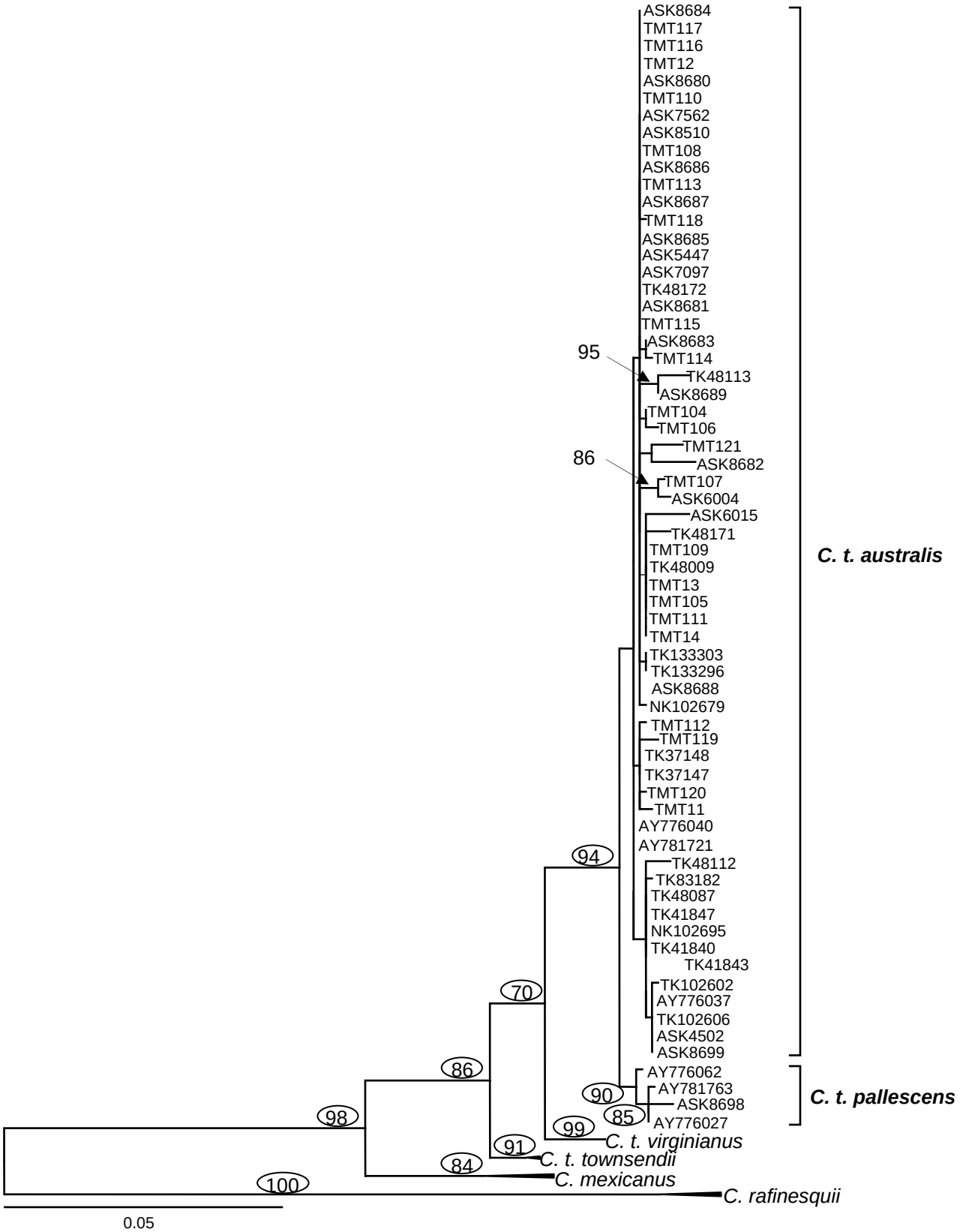

Fig. 3 — Phylogram based on Maximum likelihood analysis (HKY+G) of 951 base pairs of the cytochrome *b* gene for *Corynorhinus* species and subspecies. The tree was rooted using *Barbastella*. Support values on branches are significant bootstrap support values (≥70). Those numbers circled also had significant Bayesian posterior probabilities (≥0.95). Samples beginning with AY were downloaded from GenBank. All other specimen prefixes are defined in Appendix I.

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Table 1 — Diagnostic characteristics of *Corynorhinus townsendii* and *Corynorhinus mexicanus* (Handley 1959, Jones 1977)

	Corynorhinus townsendii	***Corynorhinus mexicanus***
Length of skull	≥15.7 mm (♀) ≥15.5 mm (♂)	<15.7 mm (♀) <15.5 mm (♂)
Maxillary Toothrow	≥4.9 mm	<4.9 mm
Tragus Length	≥13 mm	<13 mm
Number of Interfemoral Crossbars	≥ 9	< 9
Incisor	Secondary cusp absent	Secondary cusp present

Table 2: Summary of the morphological measurements taken during this study from *Corynorhinus*. The top number is the average measurement length with standard deviation, followed by the range (minimum-maximum) underneath. Abbreviations for measurements are defined in the text. Northern West Texas is comprised of samples from Culberson Co., TX and Otero Co., NM; Mid-West Texas is comprised of specimens from Jeff Davis Co., TX; Southern West Texas is comprised of samples from Brewster Co., Presidio Co., and Val Verde Co., TX; specimens from Mexico are from the states of Coahuila and Chihuahua.

	C. townsendii Northern West Texas (N=7)	*C. townsendii* Mid-West Texas (N=8)	*C. townsendii* Southern West Texas (N=44)	*C. townsendii* Mexico (N=5)
TL	16.24±0.44	15.81±0.19	15.83±0.33	15.81±0.06
	(15.69-16.83)	(15.67-16.22)	(15.22-16.65)	(15.74-15.88)
ZB	8.73±0.34	8.38±0.20	8.33±0.27	8.52±0.24
	(8.34-8.93)	(8.21-8.6)	(7.48-8.83)	(8.26-8.73)
CB	7.59±0.27	7.4±0.10	7.43±0.23	7.44±0.12
	(7.27-7.96)	(7.31-7.56)	(6.68-7.78)	(7.34-7.61)
MB	8.43±0.36	8.65±0.25	8.69±0.32	8.7±0.12
	(7.87-8.88)	(8.17-8.91)	(8.09-9.27)	(8.53-8.81)
POC	3.42±0.08	3.36±0.13	3.38±0.11	3.38±0.09
	(3.33-3.52)	(3.24-3.6)	(3.15-3.61)	(3.31-3.5)
MT	4.9±0.13	4.81±0.12	4.77±0.25	4.9±0.09
	(4.71-5.07)	(4.61-4.94)	(3.76-5.07)	(4.83-5.04)
PL	5.3±0.30	5.15±0.30	5.29±0.13	5.41±0.12
	(4.71-5.69)	(4.46-5.45)	(5.00-5.34)	(5.24-5.51)
BL	11.46±0.27	11.18±0.16	11.35±0.41	11.27±0.17
	(11.11-11.91)	(10.95-11.44)	(10.59-12.5)	(11.05-11.41)
ABL	3.98±0.12	3.78±0.09	3.82±0.13	3.89±0.16
	(3.77-4.11)	(3.68-3.93)	(3.49-4.03)	(3.66-4.03)

Table 2 Continued

ICW	2.7±0.13	2.59±0.26	2.39±0.25	2.53±0.32
	(2.58-2.96)	(2.23-2.92)	(1.97-2.86)	(2.04-2.74)
PBM3	5.6±0.16	5.54±0.12	5.63±0.16	5.69±0.07
	(5.36-5.78)	(5.42-5.76)	(5.34-6.05)	(5.61-5.77)
IPW	2.25±0.13	2.2±0.14	2.14±0.13	2.13±0.16
	(2.11-2.44)	(2.04-2.46)	(1.92-2.51)	(1.94-2.22)
CD	4.92±0.36	5.06±0.43	4.9±0.43	4.73±0.50
	(4.49-5.45)	(4.25-5.68)	(3.7-5.66)	(4.00-5.05)
DL	10.14±0.29	10±0.19	10±0.24	10.09±0.17
	(9.77-10.6)	(9.77-10.28)	(9.51-10.43)	(9.89-10.32)
MAT	2.66±0.26	2.61±0.14	2.52±0.15	2.41±0.09
	(2.31-2.99)	(2.43-2.8)	(2.28-2.79)	(2.28-2.5)
MAM	2.18±0.15	2.16±0.11	2.11±0.17	2.13±0.10
	(1.98-2.36)	(2.02-2.32)	(1.74-2.39)	(2.02-2.23)
CA	3.67±0.15	3.66±0.08	3.59±0.15	3.71±0.11
	(3.55-3.93)	(2.53-3.74)	(3.28-3.88)	(3.55-3.83)
FC	2.37±0.23	2.51±0.12	2.37±0.19	2.23±0.15
	(2.06-2.64)	(2.4-2.68)	(2.05-2.78)	(2.03-2.37)

Table 3: Summary of *Corynorhinus townsendii* specimens from West Texas and the type (*C. townsendii*-like or *C. mexicanus*-like) of characteristics they possess.

	Total number evaluated	*Corynorhinus townsendii*-like	*Corynorhinus mexicanus*-like	Percentage with *C. mexicanus* characteristics*
Length of skull	61	48	13	21%
Maxillary Toothrow	60	38	22	37%
Tragus Length	27	20	7	26%
Number of Interfemoral Crossbars	60	46	14	23%
Incisor	65	50	15	19%

*19 of these specimens shared two or more *C. mexicanus*-like characteristics

Appendix I — Species, locality, tissue and catalog numbers for specimens used in the morphological analysis (M). Genbank numbers signify those specimens used in the cytochrome *b* molecular analysis (GenBank number). ASK (tissue number) and ASNHC (catalog number) = Angelo State Natural History Collections, Angelo State University; NK (tissue number) and MSB (catalog number) = Museum of Southwestern Biology, University of New Mexico; and TK (tissue number) and TTU (catalog number) = Natural Science Research Laboratory, Texas Tech University. Tissues with only a single field number (TMT) were wing punches collected in the field.

Corynorhinus townsendii –**Mexico: Coahuila, 6 mi S Cuatro Cienegas**, TTU93474 (M), TTU93475 (M), TTU93476 (M), TTU93477(M); **Chihuahua, 40 mi SE Ojinaga**, ASNHC 1049 (M)

United States: New Mexico; Otero Co., 3.5 mi E Lightning Lake, TTU 7473 (M)

United States: Texas; Brewster Co., ASK8699 (JQ916965) **River Mile 723 Rio Grande**, ASK 6004, ASNHC 11586 (M, JQ916949); **Big Bend National Park, Boot Spring Drainage**, ASK 7562, ASNHC 13284 (M, JQ916952); TMT 120 (JQ917002); TMT 121 (JQ917003); **Big Bend National Park, Emory Cave**, ASK 7097, ASNHC 13285 (M, JQ916951); **Big Bend National Park, Ernst Tinaja**, ASK 8716, ASNHC 14324 (M); ASK 8717, ASNHC 14325 (M); **Big Bend National Park, Glenn Springs**, ASK 8510, ASNHC 13388 (M, JQ916953); TMT 118 (JQ916999); TMT 119 (JQ917000); **Big Bend National Park, Glenn Spring Runoff**, ASK 6015, ASNHC 11585 (M, JQ916950); **Big Bend National Park, Harte Ranch, 0.5 mi S, 1.5 mi E Key Place**, TTU 60277 (M); **Big Bend National Park, Mariscal Mine**, ASK 8550, ASNHC 14313 (M); ASK 8680, ASNHC 14314 (M, JQ916954); ASK 8683, ASNHC 14315 (JQ916957); ASK 8684, ASNHC 14316 (M,

JQ916958); ASK 8685, ASNHC 14317 (M, JQ916959); ASK 8687, ASNHC 14318 (M, JQ916961); ASK 8681, ASNHC 14319 (M, JQ916955); ASK 8688, ASNHC 14320 (M, JQ916962); ASK 8682, ASNHC 14321 (M, JQ916956); ASK 8686, ASNHC 14322 (M, JQ916960); ASK 8689, ASNHC 14323 (M, JQ916963); TMT11 (JQ916990); TMT12 (JQ917001); TMT 13 (JQ917004); TMT 14 (JQ917005); TMT 104 (JQ916984); TMT 105 (JQ916985); TMT 106 (JQ916986); TMT 107 (JQ916987); TMT 108 (JQ916988); TMT 109 (JQ916989); TMT 110 (JQ916991); TMT 111 (JQ916992); TMT 112 (JQ916993); TMT 113 (JQ916994); TMT 114 (JQ916995); TMT 115 (JQ916996); TMT 116 (JQ916997); TMT 117 (JQ916998); **0.25 mi N Rio Grande, San Francisco Canyon**, ASK 5447, ASNHC 11525 (JQ916948); **Black Gap Wildlife Management Area**, NK 37148, MSB 92633 (M, JQ916973); TTU 79331 (M).

United States: Texas; Culberson Co., Guadalupe Mountains National Park, Manzanita Springs, TTU 19957 (M); **Guadalupe Mountains National Park, The Bowl (Earthen Tank)**, TTU 19958 (M); TTU 19960 (M); TTU 19961 (M); **Guadalupe Mountains National Park, Lost Peak Mine**, TTU 23277 (M); **Guadalupe Mountains National Park, Upper Dog Ranger Station**, TTU 23281 (M).

United States: Texas; Hutchinson Co., ASK8698 (JQ916964)

United States: Texas; Jeff Davis Co., Davis Mountains Preserve Headquarters, NK 102602, MSB 99240 (M, JQ916968); NK 102606, MSB 99242 (M, JQ916969); **Davis**

Mountains Upper Madera Crossing, NK 102679, MSB 99299 (M, JQ916966); NK 102695, MSB 99304 (M, JQ916967); **3.5 mi NE Fort Davis**, TTU 14068 (M); **17 mi W Fort Davis**, TTU 17232 (M); **3 mi E junction of highways 166 and 505, Harris Hole, Bryan Harris Ranch**, TTU 9148 (M); **CA 8 mi S junction of highways 166 and 118, Davis Mountains**, TTU 9161 (M).

United States: Texas; Presidio Co., 19 mi N Presidio, ASNHC 748 (M); ASNHC 749 (M); ASNHC 750 (M); **ZH Canyon, Clay Miller Ranch, Sierra Vieja Mountains**, ASK 4502, ASNHC 9566 (M, JQ916947); **10 mi W Valentine, ZH Canyon, C. E. Miller Ranch**, TK 13285, TTU 34550 (M); TK 13253, TTU 34551 (M); TTU 34552 (M); **Big Bend Ranch State Natural Area**, TK 41840, TTU 67171 (M, JQ916974); TK 41843, TTU 67172 (M, JQ916975); TK41847, TTU 67173 (M, JQ916976); TTU 67174 (M); TK 48087, TTU 68380 (M, JQ916978); TK 48112, TTU 68381 (M, JQ916979); TK 48113, TTU 68382 (M, JQ916980); TK 48171, TTU 68383 (M, JQ916981); TK 48172, TTU 68384 (M, JQ916982); TK 48009, TTU 67722 (JQ916977); TTU 69630 (M); **10 mi. WSW Valentine, Clay Miller Ranch**, TK 83182, TTU 78531 (M, JQ916983).

United States: Texas; Val Verde Co., 4 mi S, 2 mi W Langtry, Rattlesnake Canyon, TK133296, TTU 111185 (M, JQ916970); TK 133303, TTU 111186 (M, JQ916971); **3 mi S, 3.5 mi W Langtry**, TTU 47108 (M); **Fisher's Fissure, 2 mi W Langtry**, TTU 6547 (M); TTU 6548 (M); **33 mi N, 6 mi E Del Rio**, TTU 6921 (M); **Evert Canyon, 20 mi NW Comstock**, TTU 93450 (M).

Corynorhinus mexicanus **– Mexico, Nuevo Leon, 1 km S Ejido San Josecito, Cueva San Josecito**, TK 32525, TTU 57065 (M).

Appendix II — List of GenBank sequences from *Corynorhinus* used in this study.

GenBank Number	Species Name	Source
AY776040	*C. t. australis*	Piaggio and Perkins (2005)
AY781721	*C. t. australis*	Piaggio and Perkins (2005)
AY776037	*C. t. australis*	Piaggio and Perkins (2005)
AY776062	*C. t. pallescens*	Piaggio and Perkins (2005)
AY781763	*C. t. pallescens*	Piaggio and Perkins (2005)
AY776027	*C. t. pallescens*	Piaggio and Perkins (2005)
AY776075	*C. t. virginianus*	Piaggio and Perkins (2005)
AY781774	*C. t. virginianus*	Piaggio and Perkins (2005)
AY781734	*C. t. townsendii*	Piaggio and Perkins (2005)
AY781714	*C. t. townsendii*	Piaggio and Perkins (2005)
AY781716	*C. t. townsendii*	Piaggio and Perkins (2005)
AY776067	*C. mexicanus*	Piaggio and Perkins (2005)
AY776038	*C. mexicanus*	Piaggio and Perkins (2005)
AY776039	*C. mexicanus*	Piaggio and Perkins (2005)
AY776041	*C. mexicanus*	Piaggio and Perkins (2005)
AY781775	*C. rafinesquii*	Piaggio and Perkins (2005)
AY776074	*C. rafinesquii*	Piaggio and Perkins (2005)
AY781728	*C. rafinesquii*	Piaggio and Perkins (2005)
EF534761	*Barbastella beijinensis*	Zhang et al. (2007)
EF534766	*Barbastella leucomelas*	Zhang et al. (2007)