GENETIC AND MORPHOLOGIC VARIATION IN THE DAVIS MOUNTAINS COTTONTAIL (*SYLVILAGUS ROBUSTUS*)

Amy V. Nalls,* Loren K. Ammerman, and Robert C. Dowler

Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80525 (AVN)
Department of Biology, Angelo State University, San Angelo, TX 76909 (LKA, RCD)
**Correspondent: amy.nalls@colostate.edu*

Abstract—The Davis Mountains cottontail, *Sylvilagus robustus*, is an endemic species of the Trans-Pecos area of Texas. Although *S. robustus* previously was believed to be extirpated from the Chisos Mountains, we confirmed existence of a population. We examined intrapopulation and interpopulation variation in *S. robustus*, as well as the genetic relationship to the eastern cottontail (*S. floridanus*) using partial sequences of the mitochondrial cytochrome *b* and control region genes. Six morphometric traits relating to overall size and six cranial characters considered diagnostic for the two subspecies were used to confirm identification of specimens. Our morphological analysis suggested that specimens from the Chisos and Davis Mountains were *S. robustus*; however, low levels of genetic divergence between *S. robustus* and *S. floridanus* appeared inconsistent with species-level recognition for *S. robustus*.

Resumen—El conejo del monte, *Sylvilagus robustus*, es una especie endémica de la zona de Trans-Pecos de Texas. Aunque *S. robustus* fue anteriormente considerada erradicada de las montañas Chisos, confirmamos la existencia de una población. Examinamos la variación genética dentro y entre poblaciones en *S. robustus*, y también la relación genética con el conejo de castilla (*S. floridanus*) usando secuencias parciales de genes de ADN mitocondriales de la región de control y de citocromo *b*. Seis características morfométricas relacionadas con el tamaño corporal y seis características de cráneos consideradas diagnósticas para las dos subespecies fueron usadas para confirmar la identificación de los especímenes. Nuestro análisis morfológico sugirió que los especímenes colectados de las montañas Chisos y Davis fueron *S. robustus*; sin embargo, los bajos niveles de divergencia genética entre *S. robustus* y *S. floridanus* parecieron inconsistentes con el reconocimiento del nivel de especie para *S. robustus*.

The Davis Mountains cottontail, *Sylvilagus robustus*, is an endemic species of the Trans-Pecos region of Texas, occurring in pinyon-oak-juniper (*Pinus-Quercus-Juniperus*) woodlands of the Guadalupe, Davis, Chinati, and Chisos Mountains at elevations of 1,432–2,438 m (Schmidly, 1977). *Sylvilagus robustus* is larger and darker than the widely distributed eastern cottontail, *S. floridanus* (Schmidly, 1977). Specimens also are known from Coahuila, Mexico, originally described as *S. floridanus nelsoni* by Baker (1955) but synonymized with *S. robustus* by Raun (1965). Reports, but no specimen, of this cottontail were obtained in the Sierra del Carmen of northern Mexico (Baker, 1956).

Sylvilagus robustus was first described as *Lepus pinetis robustus* from specimens collected by V. Bailey at an elevation of 1,829 m in the Fort Davis area of Jeff Davis County, Texas (Bailey, 1905). *Sylvilagus robustus* was elevated to species level by Nelson (1909) on the basis of morphological features and lack of apparent intergrades, and it maintained this status until Hall and Kelson (1951) relegated the taxon to a subspecies of *S. floridanus* based on intermediate morphology of one specimen (Louisiana State University Museum of Natural Science 658) between *S. f. cognatus* and *S. robustus*. Davis (1974) gave an account of this taxon as *S. robustus*. Schmidly (1977) considered it a subspecies after examining specimens of *S. floridanus* from throughout Texas and New Mexico and observing only subspecific-level differences in *robustus*. Subsequently, Davis and Schmidly (1994:92) cited "nominal cranial differences with *S. floridanus*" as the basis for their subspecific designation of *robustus*. However, Ruedas (1998) conducted a multivariate morphological analysis of 26 cranial, mandibular, and dental characters examining five taxa of cottontails (*S. floridanus chapmani*, *S. f. cognatus*, *S. f. holzneri*, *S. f. robustus*, and *S. nuttallii pinetis*) and detected a separation of group means for six cranial characters between *robustus* and all other taxa examined (*robustus* having the largest means). Ruedas (1998) also reported differences in discrete cranial and mandibular characters in specimens of *robustus* and the parapatric *S. floridanus chapmani*. Schmidly (2004) followed this taxonomy, recognizing *S. robustus* as a species.

Collection of Davis Mountains cottontails has proven

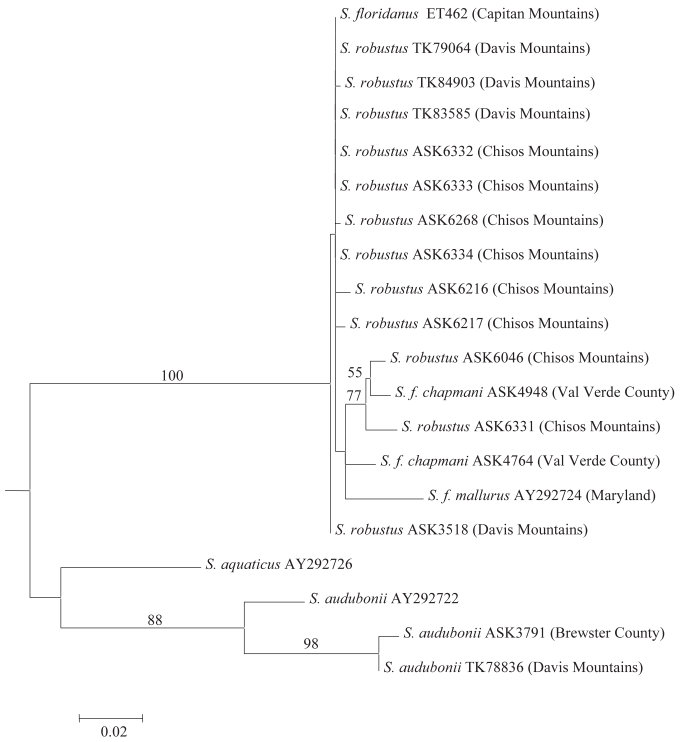

Fig. 1—Results of the maximum-likelihood analysis (HKY+Γ) based on sequences of cytochrome *b* of *Sylvilagus*. Bootstrap values >50 based upon 100 replicates are shown above branches.

difficult in the past because of small populations that are presumably a result of low precipitation and productivity in the montane areas they inhabit (Ruedas, 1998). Prior to this study, no specimen was known from the Chisos or Guadalupe Mountains within the past 30 years (Ruedas, 1998). Schmidly (2004) reported a recent collection of several specimens from the Davis Mountains indicating that a healthy population remained there.

There currently are no published mitochondrial data examining *S. robustus* as a taxon, or its relationship to the parapatric *S. floridanus chapmani*, but morphological differences suggest some level of separation from *S. floridanus*. We examined variation in sequences across portions of the control region and cytochrome *b* gene in the mitochondrial-DNA genome to determine genetic relationships between *S. robustus* and *S. floridanus*, levels of gene flow between isolated populations of *S. robustus*, and genetic variation within populations. We also recorded morphological data for *S. robustus* and *S. floridanus* and compared these to results of Ruedas (1998).

Materials and Methods—We sampled cottontails in the Chisos Mountains of Big Bend National Park, Brewster County, Texas, at elevations of 1,470–2,027 m during May 2003–August 2004 and in Lincoln National Forest, Guadalupe Mountains, Eddy County, New Mexico, at elevations of 1,806–2,164 m during October 2003–January 2004. We trapped for a total of 485 trapnights (one trapnight equals one trap set for one night) in Big Bend National Park and 154 trapnights in the Guadalupe Mountains. Specimens of *Sylvilagus* that were dead on the road in Big Bend National Park were salvaged if they were above 1,220 m elevation. We baited live traps with apples and alfalfa, as suggested by Forys and Humphrey (1997). We identified specimens as *S. robustus* if they were collected above the known lower-elevational limit for the species (1,432 m), and external measurements were consistent with those reported for the species (Schmidly, 2004). Samples of heart, liver, kidney, and muscle tissues were taken (when present) from each individual collected from Big Bend National Park and frozen for preservation. Voucher specimens and tissues were deposited in the Angelo State Natural History Collections (ASNHC). We included additional specimens from the Davis Mountains and southern New Mexico (Appendix) deposited in the collections at Eastern New Mexico University, Texas Tech University, and the Angelo State Natural History Collections.

We isolated total genomic DNA from liver, heart, muscle, or lung tissues using a DNeasy Tissue Kit (QIAGEN, Inc., Valencia, California) following protocol of the manufacturer. We amplified partial sequences of cytochrome *b* using universal primers L14841 (Kocher et al., 1989) and H15547 (Edwards et al., 1991), whereas partial sequences of the control region were generated using primers designed for chiropterans (e.g., CRP-L and CRF-H; Wilkinson and Chapman, 1991).

Samples were amplified using an initial denaturing period at 93°C for 3 min, followed by 39 cycles of amplification (denaturation at 94°C, annealing at 48°C, and extension at 72°C for 1 min each), followed by a final extension period at 72°C for 3 min. PCR products were purified using low-melt agarose gels (1.0% w/v) premixed with 0.05% (10 ug/mL) ethidium bromide, then cloned using the TOPO TA Cloning Kit (Invitrogen Corporation, Carlsbad, California) following protocol of the manufacturer (modified such that only one-fourth of the recommended volume was used). Plasmids containing PCR product were isolated with QIAprep Spin Miniprep Kit (QIAGEN, Inc., Valencia, California) and DNA sequenced using USB ThermoSequenase Cycle Sequencing Kit (United States Biochemical Corp., Cleveland, Ohio) and fluorescent dye-labeled primers (M13 forward [-29] IRDye 700 and M13 Reverse IRDye 800; Licor Biosciences, Lincoln, Nebraska). Sequences were analyzed on a Licor NEN Global IR2 DNA analyzer system using eSeq software (versions 2.0 and 3.0). We aligned sequences using the program Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan) and compared to the following sequences of cottontails obtained from GenBank: *S. floridanus mallurus* (AY292724), *S. audubonii* (AY292722), and *S. aquaticus* (AY292726). *Sylvilagus audubonii* was used as the outgroup because it occupies a basal position to *S. floridanus* in the phylogeny of *Sylvilagus* (Halanych and Robinson, 1997). *Sylvilagus aquaticus* was included for additional comparison of specific-level, genetic distance.

The best-fit model of evolution of sequences of DNA for likelihood analysis was determined using MODELTEST version 3.06 (Posada and Crandall, 1998). Standard phylogenetic analyses using the maximum-likelihood method (Felsenstein, 1985) were conducted using PAUP* version 4.0b10 (Swofford, 2001). Statistical support of specific nodes was determined through likelihood bootstrap analyses (Felsenstein, 1985) using 100 replicates. Branches with bootstrap proportions >70% were considered well-supported (Hillis and Bull, 1993). For cytochrome *b* data, we calculated pair-wise divergence of sequences

TABLE 1—Average Kimura two-parameter pair-wise mitochondrial sequence divergence (%) for *Sylvilagus*.

Comparison	Cytochrome *b*		Control region	
	Mean (*n*)	Range	Mean (*n*)	Range
Sylvilagus robustus to *S. floridanus*	2.30 (12, 3)	1.09–3.99	3.64 (22, 5)	1.79–5.86
Sylvilagus robustus to *S. f. chapmani*	1.64 (12, 2)	1.09–2.21	3.71 (22, 4)	1.79–5.86
Sylvilagus robustus to *S. f. mallurus*	2.96 (12, 1)	2.69–3.99	—	—
Sylvilagus robustus to *S. f. alacer*	—	—	3.57 (22, 1)	2.30–4.42
Within *Sylvilagus f. chapmani*	2.38 (2)	—	4.15 (4)	1.82–6.15
Within *Sylvilagus robustus*	0.76 (12)	0–2.20	1.41 (22)	0–4.43
Within Davis Mountains *robustus*	0.15 (4)	0–0.31	1.17 (11)	0–2.30
Within Chisos Mountains *robustus*	1.10 (8)	0–2.20	1.66 (11)	0–4.72
Davis to Chisos Mountains *robustus*	0.63	0–2.04	1.43	0.50–4.17
Capitan Mountains to *robustus*	0.39 (1, 12)	0–1.88	0.81 (1, 12)	0–3.90
Sacramento Mountains to *robustus*	—	—	1.21 (1, 12)	0.25–4.17
Otero County (canyon) to *robustus*	—	—	2.90 (1, 12)	2.04–3.62
Sylvilagus floridanus to *S. audubonii*	14.30 (3, 3)	13.33–15.40	14.94 (5, 6)	13.53–16.24
Sylvilagus floridanus to *S. aquaticus*	11.48 (3, 1)	11.00–11.77	—	—
Sylvilagus audubonii to *S. aquaticus*	11.14 (3, 1)	9.97–11.82	—	—

under the Kimura two-parameter model of evolution (Kimura, 1980) to facilitate comparison with divergences between pairs of species as in Bradley and Baker (2001).

We took actual measurements (when the carcass was intact) or used data from labels of museum specimens including total length, length of tail, length of hind foot, and length of ear as described by Schmidly (2004), as well as mass and gender for adult *S. robustus* and *S. floridanus*. We also measured six cranial characters reported by Ruedas (1998) to separate *robustus* from five other taxa of cottontails (i.e., greatest length of skull, condylopremaxillary length, breadth of rostrum, interbasioccipital length, width of auditory bullae, and mastoid breadth). All measurements were taken to the nearest 0.1 mm using digital calipers. We calculated mean, standard deviation, and range for *robustus* and *chapmani* and compared our results to data in Ruedas (1998). We also scored discrete mandibular and cranial characters identified by Ruedas (1998) to distinguish *robustus* and *chapmani*, including the basisphenoid foramina, tympanic process, and mental foramen.

RESULTS—Ten road-killed individuals were salvaged from within Big Bend National Park at elevations of 1,463–1,768 m, and only one was captured in a trap (ASNHC 12940). Collecting trips in the Guadalupe Mountains were unsuccessful. A list of individuals and collecting localities is provided in the Appendix.

We sequenced a total of 648 base pairs of the cytochrome-*b* gene for eight individuals from the Chisos Mountains, three from the Davis Mountains, one *S. floridanus* from the Capitan Mountains, two *S. f. chapmani*, and two *S. audubonii* (Genbank numbers HQ143448–HQ143464).

Average divergence of sequences between *S. robustus* and *S. f. chapmani* was low (1.64%), ranging from 1.09 to 2.21% (Table 1). Average divergence among all *S. robustus* was 0.76%, while within the Davis Mountains it was 0.15% and for Chisos Mountains it was 1.08%. Divergence between Davis and Chisos mountains averaged 0.63%. The individual from the Capitan Mountains differed from *S. robustus* by an average of 0.39%. Divergence of *S. floridanus* from *S. audubonii* and *S. aquaticus* averaged 14.30 and 11.48%, respectively.

Best-fit evolutionary model for the cytochrome *b* data was identified as HKY+Γ (Hasegawa et al., 1995), resulting from both hierarchical likelihood-ratio tests (hLRTs) and Akaike Information Criterion (minimum-theoretical-information criterion, AIC; Akaike, 1974). The dataset contained the following base frequencies: A = 0.2578, C = 0.3185, G = 0.1362, T = 0.2874. The transition-to-transversion ratio was 7.869. A single tree was produced in PAUP* (Swofford, 2001) under maximum-likelihood criteria (−Ln = 1,752.500; Fig. 1). Individuals of *S. robustus* and *S. floridanus* were combined into a single monophyletic clade supported by a bootstrap proportion of 100%. *Sylvilagus audubonii* formed a clade supported by a bootstrap proportion of 88%.

For the control region, we sequenced a total of 430 base pairs for 11 individuals from the Chisos Mountains; 11 from the Davis Mountains; four *S. f. chapmani*, one *S. f. alacer*, and one *S. floridanus* from the Capitan Mountains; one *S. floridanus* from the Sacramento Mountains; one *S. floridanus* from Otero County; and six *S. audubonii* (Genbank numbers HQ143412–HQ143447). A hypervariable region of 30 bases that could not be unambiguously aligned was excluded from the analyses, leaving a total of 400 characters for phylogenetic analysis.

When sequence of the control region was compared for all *S. robustus*, divergences of 0–4.43% were detected (Table 1). Within populations in the Davis and Chisos mountains, divergences were 0–2.30 and 4.72%, respectively, while a range of 0.50–4.17% was detected between these isolated populations. Divergence averaged 3.71% between *S. robustus* and the parapatric *S. f. chapmani*, and 3.57% between *S. robustus* and the allopatric *S. f. alacer*.

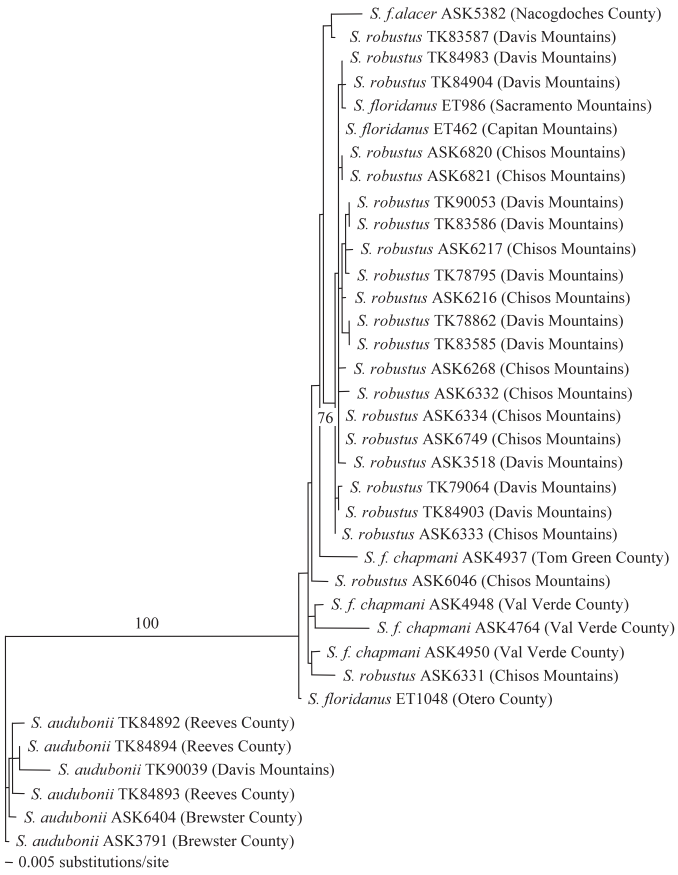

FIG. 2—Results of the maximum-likelihood analysis (TVM+Γ) based on sequences in the control region of *Sylvilagus*. Bootstrap values >70 based upon 100 replicates are shown above branches.

Divergence averaged 14.58% between *S. floridanus* (*chapmani* and *alacer* combined) and *S. audubonii*.

For the control-region dataset, MODELTEST (Posada and Crandall, 1998) suggested the HKY+ Γ model under the hLRTs and the TVM+Γ model under AIC. The dataset contained the following base frequencies: A = 0.3427, C = 0.0912, G = 0.2599, T = 0.3062. Transition-to-transversion ratio was 3.928. A single tree was produced in PAUP* under maximum-likelihood criteria (−Ln = 1,534.474; Fig. 2). As in the cytochrome *b* data, *S. robustus* and *S. floridanus* formed a monophyletic clade strongly supported by a bootstrap proportion of 100%. No distinct lineage was identified for either Davis or Chisos mountains. A monophyletic clade with significant bootstrap support was recovered including 19 *S. robustus*, one individual from the Capitan Mountains, and one individual from the Sacramento Mountains (Fig. 2). The six *S. audubonii* formed a monophyletic clade outside the *S. robustus*-*S. floridanus* clade.

Average measurements of adult *S. robustus* in our study ($n = 18$) were consistent with those reported by Schmidly (2004), although measurements of total length and length of tail were highly variable among individuals as were measurements of total length, length of tail, and length of ear in *S. floridanus* ($n = 5$). Average measurements and mass reported for *S. robustus* and *S. floridanus* in Texas (Schmidly, 2004) were as follows, respectively: total length, 416 and 418 mm; length of tail, 53 and 56 mm; length of hind foot, 98 and 92 mm; length of ear, 71 and 52 mm; mass, 1.3–1.8 and 1–2 kg. Average measurements and mass for *S. robustus* and *S. floridanus* in our study were, respectively: total length, 413 ($SD = 34$) and 369 mm ($SD = 34$); length of tail, 57 ($SD = 10$) and 41 mm ($SD = 6$); length of hind foot, 99 ($SD = 4$) and 87 mm ($SD = 4$); length of ear, 77 ($SD = 3$) and 66 ($SD = 12$); mass, 1.12 ($n = 10$; $SD = 0.25$) and 0.8 kg ($n = 5$; $SD = 0.2$). Unpaired *t*-tests revealed significant differences between adults of *S. robustus* and *S. floridanus* in our study for total length ($P = 0.022$), length of tail ($P < 0.001$), length of hind foot ($P < 0.001$), and mass ($P = 0.016$).

For each of the six cranial characters measured, the mean for *S. robustus* was greater than the mean for *S. f. chapmani* (Table 2), as was presented by Ruedas (1998). In contrast, discrete cranial characters examined in *S. robustus* and *S. f. chapmani* (Vestal, 2005) were incongruent with differences in characters proposed between the two taxa by Ruedas (1998). In specimens he examined, *S. robustus* had two distinct basisphenoid foramina, while *S. f. chapmani* had a single foramen. In our study not all skulls could be assessed for all characteristics, but four of 15 skulls of *S. robustus* had two distinct foramina, while the other 11 had a single basisphenoid foramen. All four skulls of *S. f. chapmani* had a single foramen. Ruedas (1998) stated that *S. robustus* lacked a tympanic process, whereas *S. f. chapmani* had a distinct process. In our study

TABLE 2—Descriptive statistics for *Sylvilagus robustus* and *S. floridanus chapmani*.

	Sylvilagus robustus			*Sylvilagus floridanus chapmani*		
Character	Mean	*SD*	Range (*n*)	Mean	*SD*	Range (*n*)
Greatest length of skull	74.07	1.20	71.8–75.9 (11)	66.63	3.23	64.2–70.3 (3)
Condylopremaxillary length	65.74	1.02	64.3–67.0 (10)	59.43	2.64	57.1–63.2 (4)
Breadth of rostrum	19.58	0.84	18.3–20.9 (13)	17.23	1.15	16.3–18.9 (4)
Interbasioccipital length	20.56	0.75	19.4–21.5 (10)	19.23	0.91	18.4–20.5 (4)
Width of auditory bullae	13.01	1.04	12.0–16.3 (15)	11.46	0.26	11.1–11.7 (4)
Mastoid breadth	31.37	1.39	30.3–35.0 (10)	27.9	1.47	26.6–29.5 (3)

eight of 17 skulls of *S. robustus* lacked the process and the remaining nine had a distinct process. All four *S. f. chapmani* had a distinct process. Ruedas (1998) also reported that the mental foramen in *S. robustus* was twice as long as high and located on the dorsad aspect of the mandible, whereas in *S. f. chapmani*, this foramen was less than one-half as long as high and usually on the labial aspect of the mandible. In our study 16 of 19 *S. robustus* with intact mandibles had *robustus*-like mental foramina, as described by Ruedas (1998), whereas three had *chapmani*-like foramina. Three of the four *S. f. chapmani* had *robustus*-like foramina, while only one had a *chapmani*-like foramen.

Discussion—Our genetic analyses identify *Sylvilagus* from the Chisos and Davis mountains as part of the *floridanus* group. Bradley and Baker (2001) calculated genetic distances based on data for cytochrome *b* sequences to determine boundaries in species of rodents and bats. Distances reported in our study between *S. robustus* and *S. floridanus* were congruent with values reported for intraspecific divergences by these authors. Further, interspecific divergences recovered between *S. floridanus* and *S. audubonii* (14.30%), *S. floridanus* and *S. aquaticus* (11.48%), and *S. audubonii* and *S. aquaticus* (11.14%) were markedly greater than those between *S. robustus* and *S. floridanus*. Halanych and Robinson (1999) reported similar divergences of cytochrome *b* between *S. floridanus* and *S. audubonii* (12.11%), *S. floridanus* and *S. aquaticus* (11.66%), and *S. audubonii* and *S. aquaticus* (7.77%). Overall, based on cytochrome *b* data, divergence observed between *S. robustus* and *S. floridanus* was unexpectedly low for a species-level comparison.

Litvaitis et al. (1997) examined genetic variation in New England (*S. transitionalis*), Appalachian (*S. obscurus*), and eastern (*S. floridanus*) cottontails in the northeastern United States and determined a difference in sequence of the control region of 16% between *S. floridanus* and *S. transitionalis-S. obscurus* groups. Additionally, their phylogenetic analysis revealed a clear separation of eastern and New England-Appalachian cottontails, but no distinct geographic pattern was seen between New England-Appalachian cottontails or among the 46 eastern cottontails examined from 10 northeastern states. Similarly, in the present study, no resolution was seen between populations of *S. robustus* in the Chisos and Davis mountains in either cytochrome *b* or control region. Divergences in the control region between the two isolated populations (0.50–4.17%) overlapped divergences within each population (Davis Mountains 0–2.30% and Chisos Mountains 0–4.72%). Branco et al. (2002) reported similar intrapopulational values for divergence of control region (0–7.72) among nine populations of the European rabbit (*Oryctolagus cuniculus*) on the Iberian Peninsula. Considering the comparatively large divergence values for cytochrome *b* and the control region previously reported between species of *Sylvilagus* (Litvaitis et al., 1997; Halanych and Robinson, 1999), the divergences we observed between *robustus* and *floridanus* are unlikely to warrant recognition of species status for *robustus*.

The low values for genetic divergence between populations in the Chisos and Davis mountains suggest that these populations have not been isolated a sufficient amount of time to cause detectable levels of genetic divergence. Considering the adjacent geographic position of the Davis and Chisos mountains, the lack of detectable divergence may be a result of the depression of the pinyon-oak-juniper zone $\geq$790 m below its present lower limits at 1,400 m on the slopes of the Chisos Mountains, which occurred during the latest Wisconsin pluvial as recently as 11,500 years ago (Wells, 1974). This depression could have provided a forested corridor that probably extended over much of the Trans-Pecos (Schmidly, 1977) and provided an opportunity for gene flow among montane populations.

No conclusion can be made as to taxonomic identity of the three individuals from southern New Mexico (e.g., Capitan and Sacramento mountains and Otero County), because their divergences to *robustus* are comparable to those both within *robustus* and between *robustus* and *floridanus*. However, the specimen from Otero County was outside the entire *robustus-floridanus* group in the maximum-likelihood tree. Also, *S. f. cognatus* has been recorded from the northeastern slope of the Capitan Mountains (Hall and Kelson, 1951), so this may be the identity of the individual in this study from Capitan Mountains. Individuals from the Sacramento Mountains and Otero County were juveniles, which prevented an accurate comparison of cranial and external measurements of these specimens to those of *S. robustus* and *S. floridanus*.

Geographic variation in size of *S. floridanus* has been examined from three climatically distinct and disjunct regions within the range of the species: northeastern United States and southern Canada, southeastern United States, and southwestern United States and northern Mexico. Olcott and Barry (2000) examined 10 cranial measurements from 943 specimens from the three distinct regions and reported that variability in size was high within each of these regions, with size varying positively with elevation in the southwestern United States and northern Mexico. These results are consistent with our study, with high variability in size evident in *S. floridanus*, but consistently larger averages in cranial and external measurements in *S. robustus* that inhabit higher elevations in the region.

Although our sample of *S. f. chapmani* was small ($n = 4$), results of analysis of discrete cranial characters of *S. robustus* and *S. f. chapmani* were incongruent with differences proposed between the two taxa by Ruedas

(1998). Of the 14 *S. robustus* examined for all three discrete characters, only one (from the Chisos Mountains) possessed *robustus*-like forms for all three characters, as described by Ruedas (1998). Of the four *S. f. chapmani* examined for all three discrete characters, only one possessed *chapmani*-like forms for all three characters, as described by Ruedas (1998). These discrete characters failed to separate *S. robustus* from *S. f. chapmani*. Incongruent morphological datasets, in addition to low values for genetic divergence between *S. robustus* and *S. floridanus*, suggest that populations of *S. robustus* have not been isolated a sufficient amount of time from *S. floridanus* to cause species-level differences.

Considering recent collections in the Davis Mountains, there appears to be a healthy population of *S. f. robustus* remaining there. At least a small population exists in the Chisos Mountains, evident by road-kills and sightings. Further sampling is needed in mountain ranges spanning the Trans-Pecos and surrounding areas to better elucidate distribution of subspecies of *S. floridanus*, as well as to determine size and conservation status of these populations.

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Appendix

Species, collection locality, tissue, and catalog number for specimens used in analyses of cytochrome *b* (B), control region (C), and morphology (M). Specimens from the following institutions are included: Angelo State Natural History Collections (ASNHC, ASK), Natural Science Research Laboratory, Texas Tech University (TTU, TK), and Eastern New Mexico University (ENMU, ET).

Sylvilagus audubonii—USA: Texas: Brewster County, Black Gap Wildlife Management Area ASNHC9639 ASK3791 (B, C); Big Bend National Park, 5 miles N Persimmon Gap on Highway 385 ASNHC12943 ASK6404 (C). Jeff Davis County, Mount Livermore Preserve TTU101635 TK78836 (B), TTU101641 TK90039 (C). Reeves County, Sandia Springs TTU81104 TK84892 (C), TTU81105 TK84893 (C), TTU81106 TK84894 (C).

Sylvilagus floridanus alacer—USA: Texas: Nacogdoches County, Alazon Bayou Wildlife Management Area ASNHC12269 ASK5382 (C, M).

Sylvilagus floridanus chapmani—USA: Texas: Val Verde County, Devil's River State Natural Area ASNHC10795 ASK4764 (B, C, M), ASHNC11051 ASK4948 (B, C, M), ASNHC11064 ASK4950 (B, C, M). Tom Green County, San Angelo State Park ASNHC11974 ASK4937 (C, M).

Sylvilagus floridanus—USA: New Mexico: Lincoln County, Capitan Mountains, Head of Corral Canyon, elevation 1,479 m ENMU10664 ET462 (B, C, M). Otero County, Sacramento Mountains, James Canyon Campground, elevation 2,087 m ENMU11321 ET986 (C); Lower Three Mile Canyon burn ENMU11322 ET1048 (C).

Sylvilagus robustus—USA: Texas: Brewster County, Chisos Mountains, Big Bend National Park, Green Gulch Road, elevation 1,568 m ASNHC12239 ASK6046 (B, C, M); Big Bend National Park, Green Gulch Road, elevation 1,575 m ASNHC12941 ASK6216 (B, C, M); Big Bend National Park, Green Gulch Road, 13R 668269 3241909, elevation 1,482 m ASK6217 (B, C); Big Bend National Park, Green Gulch Road, 13R 666195 3239692, elevation 1,715 m ASNHC12940 ASK6268 (B, C, M); Big Bend National Park, Panther Pass, elevation 1,768 m ASNHC12942 ASK6331 (B, C, M); Big Bend National Park, Basin Road, near mile marker 4, elevation 1,638 m ASNHC12936 ASK6332 (B, C, M); Big Bend National Park, Basin Road ASNHC12938 ASK6333 (B, C, M); Big Bend National Park, Basin Road, elevation 1,737 m ASK6334 (B, C); Big Bend National Park, Basin Road, near mile marker 2 ASNHC12937 ASK6749 (C, M); Big Bend National Park, Basin Road, near mile marker 3 ASNHC12935 ASK6820 (C, M); Big Bend National Park, Basin Road, Chisos Basin Campground turnoff ASNHC12939 ASK6821 (C, M). Jeff Davis County, Davis Mountains State Park TTU7919 ASK3518 (B, C, M); Mount Livermore Preserve TTU101634 TK78795 (C), TTU101636 TK78862 (C, M), TTU101637 TK79064 (B, C, M), TTU101638 TK83585 (B, C, M), TTU101639 TK83586 (C, M), TTU101640 TK83587 (C, M), TTU81115 TK84903 (B, C, M), TTU81116 TK84904 (C, M), TTU81191 TK84983 (C, M), TTU101642 TK90053 (C, M).