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# Genetic Variation in the Davis Mountains Cottontail (*Sylvilagus robustus*) from the Chisos Mountains based on Mitochondrial DNA Sequence

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**ABSTRACT**—The Davis Mountains cottontail, *Sylvilagus robustus*, is an endemic species of the Trans-Pecos area. The status of the population is unknown, but was thought to be extirpated in the Chisos Mountains. DNA sequence from the cytochrome *b* gene was obtained from eight individuals recently collected from the Chisos Mountains. Our results suggest that the population from the Chisos Mountains is *S. robustus*. However, low levels of genetic divergence between *S. robustus* and *S. floridanus* appear inconsistent with species level status.

**RESUMEN**—El conejo conocido como Davis Mountain cottontail, *Sylvilagus robustus*, es un especie endémico en la area conocida como Trans-Pecos. El estado de la población es desconocido, pero considerado exterminado en las montañas Chisos. ADN secuencia del gen cytochrome b fue obtenida de 8 individuales recientemente colectados en las montañas Chisos. Nuestros resultados sugere que la población de las montañas Chisos es *Sylvilagus robustus*. Sin embargo, bajos niveles de divergencia genética entre *Sylvilagus robustus* y *Sylvilagus floridanus* parece ser incoherente con el nivel de estado especie.

The Davis Mountains cottontail, currently recognized as *Sylvilagus robustus*, is an endemic species of the Trans-Pecos region, found in the pinyon-oak-juniper woodlands of the Guadalupe, Davis, Chinati, and Chisos mountains at elevations between 1,433 and 2,440 m (Schmidly 1977). Reports, but no specimens, of this cottontail were also obtained in the Sierra del Carmen of northern México (Baker 1956). *Sylvilagus robustus* was first described as *Lepus pinetis robustus* from specimens collected by Vernon Bailey at an elevation of 1,830 m in the Fort Davis area of Jeff Davis County, Texas (Bailey 1905). *S. robustus* is larger in size and darker in color than the eastern cottontail, *Sylvilagus floridanus* (Schmidly 1977). *S. robustus* was elevated to species level by Nelson (1909) and maintained this status until Hall and Kelson (1951) relegated the taxon to a subspecies of *Sylvilagus floridanus* based on the intermediate morphology of one specimen between *S. f. cognatus* and *S. robustus*. In *The Mammals of Texas* (1974), W. B. Davis gave an account of this taxon as *Sylvilagus robustus*. In *The Mammals of the Trans-Pecos* (1977), Schmidly considered the taxon a subspecies after examining specimens of *floridanus* 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 a 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 (*Sylvilagus floridanus chapmani*, *S. f. cognatus*, *S. f. holzneri*, *S. f. robustus*, and *S. nuttallii pinetis*) and found that a separation of group means between *robustus* and all other cottontail taxa examined (*robustus* having the highest mean) occurred in six cranial characters. The most recent edition of *The Mammals of Texas* (Schmidly 2004) followed this taxonomy, recognizing *S. robustus* as a species.

Collection of Davis Mountains cottontails has proven difficult in the past due to small population sizes that are presumably a result of low precipitation and food productivity in the montane areas they inhabit (Ruedas 1998). Prior to this study, no specimens were known from the Chisos Mountains within the last 30 years (Ruedas 1998). There are currently no published molecular data on *S. robustus*, but morphological differences suggest some level of separation from *S. floridanus*. The objectives of this research were to establish the genetic identity of the rabbits in the Chisos Mountains and to examine intrapopulational genetic variation of *S. robustus* at this location.

Five collecting trips were made to Big Bend National Park in the Chisos Mountains between May 2003 and August 2004. Trapping efforts were conducted at elevations ranging from 1,470 to 2,027 m resulting in 485 trapnights. Live traps, baited with apple and alfalfa, were used as suggested by Forsys and Humphrey (1997). *Sylvilagus* specimens found dead on the road in Big Bend National Park were salvaged if they were located above 1,220 m in elevation. Specimens collected were identified as *S. robustus* if they were collected above *robustus*' known elevational lower limit (1,433 m), and the body measurements were consistent with those reported for the species (Schmidly 2004). Voucher specimens and tissues were deposited in the Angelo State Natural History Collections (ASNHC).

Heart, liver, and kidney tissue samples were taken from each individual and frozen for preservation. Two individuals of the *floridanus* subspecies *chapmani*, which occurs in the lowlands of central and south Texas and northern México, were included in all analyses for comparison. Total genomic DNA was extracted from the tissue and skin clips using the DNeasy Tissue Kit (QIAGEN Inc.) following the manufacturer's protocol. The cytochrome *b* gene was amplified following standard polymerase chain reaction (PCR) methods (Palumbi 1996) using a pair of primers known to work generally for vertebrates (L14841 and H15547; Kocher et al. 1989; Edwards et al. 1991). PCR products were cloned into a PCR 2.1-TOPO plasmid vector, and transformed

into *Escherichia coli* according to protocols provided with the TOPO TA Cloning Kit (Invitrogen Corporation). Plasmids containing PCR product were extracted and purified for sequencing according to the protocol of the QIAprep Spin Miniprep Kit (QIAGEN Inc.).

Sequencing reactions were performed following the manufacturer's protocol for the ThermoSequenase Cycle Sequencing Kit (USB Corp.). The reactions were run and resulting sequences analyzed on a Licor NEN Global IR2 DNA analyzer. Sequences were aligned using the program Sequencher 4.1 (Gene Codes Corporation) and manually improved through comparison to the following cottontail sequences obtained from GenBank: *Sylvilagus floridanus mallurus* (Accession No. AY292724), *Sylvilagus audubonii* (Accession No. AY292722), and *Sylvilagus aquaticus* (Accession No. AY292726). The program MODELTEST version 3.06 (Posada and Crandall 1998) was used to determine the best-fit model of evolution for likelihood analysis of the sequence data. Standard phylogenetic analyses using the maximum-likelihood (Felsenstein 1985) method were carried out using the program PAUP\* version 4.0b10 (Swofford 2001), and a bootstrap analysis was performed using 100 replicates.

Although most of the *S. robustus* specimens collected were road-killed, rendering their skulls useless, the six cranial characters that Ruedas (1998) found to separate *S. robustus* from the other cottontail taxa he examined were measured on the intact specimens of *S. robustus* and *S. floridanus* in this study.

Ten individuals were salvaged from the road within Big Bend National Park between elevations of 1,463 and 1,768 m. One individual was captured at an elevation of 1,715 m (UTM 13R 0666195,3239692), next to a fallen oak tree surrounded by hairy grama (*Bouteloua hirsuta*) and sideoats grama (*Bouteloua curtipendula*), in July 2003. A total of 648 base pairs of the cytochrome *b* gene were amplified for eight of these individuals, in addition to two *S. f. chapmani*, one *S. f. mallurus*, and two *S. audubonii*.

A single tree was produced under the criterion of maximum likelihood using the HKY+G model (Fig. 1). Branches with bootstrap values >70 were considered well-supported (Hillis and Bull 1993). The *floridanus* and *robustus* individuals formed a monophyletic clade supported by a bootstrap value of 100. The three *S. audubonii* individuals formed a separate clade supported by a bootstrap value of 99. Genetic divergence within individuals from the Chisos Mountains averaged 1.1%. The average genetic divergence was 2.0 % between *S. robustus* and *S. floridanus*, and 12.6 % between *S. robustus* and the outgroup, *S. audubonii*.

The six cranial characters that Ruedas (1998) found to separate *S. robustus* from the other cottontail taxa he examined included: greatest length of skull, condylopremaxillary

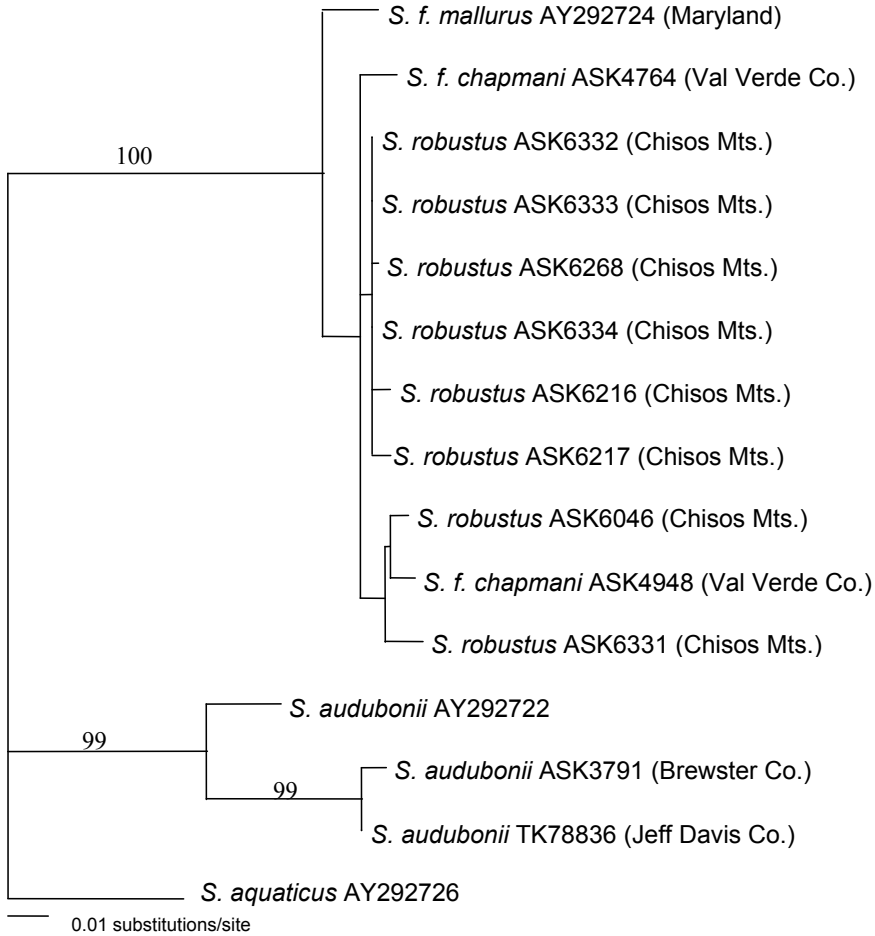


FIG. 1— Relationships of *Sylvilagus* based on maximum likelihood analysis (HKY+Γ) of cytochrome *b* sequences. Bootstrap values > 70 are shown at the branch nodes. ASK refers to the tissue collection number in the Angelo State Natural History Collections. TK refers to the specimen collection number in the Natural Sciences Research Laboratory at Texas Tech University. Specimen numbers prefixed AY were obtained from GenBank.

length, breadth of rostrum, interbasiooccipital length, width of auditory bullae, and mastoid breadth. These characters, measured in one *S. robustus* and one *S. f. chapmani* in millimeters, were 73.4, 65.2, 18.5, 20.6, 12.5, 30.5 and 65.4, 58.4, 16.7, 18.8, 11.1, 26.6, respectively. All of the measurements for *S. robustus* were greater than those for *S. f. chapmani*, as was presented by Ruedas (1998). Dental cusp patterns were generally consistent with the descriptions for *S. robustus* (Ruedas 1998), but it is clear that there is considerable variability and overlap in dentition between *S. robustus* and *S. floridanus*. Overall body size of the *S. robustus* specimens collected was consistent with that reported

for the species. The genetic results that we observed support the identification of the *Sybilagus* specimens from the Chisos as part of the *floridanus* group. The cytochrome *b* sequence divergence between the *floridanus* subspecies (*mallurus* and *chapmani*) and *robustus* are significantly lower than those between a close relative, *S. audubonii*, and *floridanus*. Similarly, Halanych and Robinson (1999) found a cytochrome *b* divergence of 12.11% between *audubonii* and *floridanus*. Based on the results of Bradley and Baker (2001) compiled for rodents and bats, a sequence divergence less than 2% as observed in this study is unlikely to merit species status. Although the sample size in this study is small, the specimens collected from the Chisos Mountains above 1,463 m appear to be *S. robustus* as recognized by Ruedas (1998) and Schmidly (2004) conflicting with Ruedas' (1998) proposition that the population was extirpated. Populations of *S. robustus* in the Davis Mountains appear healthy (Schmidly 2004) and are likely genetically isolated from other mountaintop populations. Additional study will be necessary to address the taxonomic status of *S. robustus* and to examine genetic divergence between the Davis and Chisos mountain populations.

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#### LITERATURE CITED

- BAILEY, V. 1905. Biological survey of Texas. *North American Fauna* 25:1–222.
- BAKER, R.H. 1956. *Mammals of Coahuila, Mexico*. University of Kansas Publications, Museum of Natural History 9:1–335.
- BRADLEY, R.D., AND R.J. BAKER. 2001. A test of the genetic concept: cytochrome-*b* sequences and mammals. *Journal of Mammalogy* 82:960–973.
- DAVIS, W.B. 1974. *The Mammals of Texas*. Bulletin 41, Texas Parks and Wildlife Department, Austin, Texas.
- DAVIS, W.B., AND D.J. SCHMIDLY. 1994. *The Mammals of Texas*. Texas Parks and Wildlife Department, Austin, Texas.
- EDWARDS, S.V., P. ARCTANDER, AND A.C. WILSON. 1991. Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proceedings of the Royal Society of London, Series B* 243:99–107.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. *Evolution* 39:783–791.
- FORYS, E.A., AND S.R. HUMPHREY. 1997. Comparison of 2 methods to estimate density of an endangered lagomorph. *Journal of Wildlife Management* 61:86–92.

- HALANYCH, K.M., AND T.J. ROBINSON. 1999. Multiple substitutions affect the phylogenetic utility of cytochrome *b* and 12S rDNA: Examining a rapid radiation in Leporidae (Lagomorpha) evolution. *Journal of Molecular Evolution* 48:369–379.
- HALL, E.R., AND K.R. KELSON. 1951. Comments on the taxonomy and geographic distribution of some North American rabbits. *University of Kansas Publications, Museum of Natural History* 5:49–58.
- HILLIS, D.M., AND J.J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42:182–192.
- KOCHER, T.D., W.K. THOMAS, A. MEYER, S.V. EDWARDS, S. PAABO, F.X. VILLIBLANCA, AND A.C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86:6196–6200.
- NELSON, E.W. 1909. The rabbits of North America. *North American Fauna* 29:1–314.
- PALUMBI, S. 1996. Nucleic acids II: The polymerase chain reaction. In: D.M. Hillis, C. Moritz, and B.K. Mable, editors. *Molecular Systematics*. 2<sup>nd</sup> ed. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts. Pages 205–246.
- POSADA, D., AND K.A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- RUEDAS, L.A. 1998. Systematics of *Sylvilagus* Gray, 1867 (Lagomorpha: Leporidae) from southwestern North America. *Journal of Mammalogy* 79:1355–1378.
- SCHMIDLY, D.J. 1977. *The Mammals of Trans-Pecos Texas*. Texas A&M University Press. College Station.
- SCHMIDLY, D.J. 2004. *The Mammals of Texas*. University of Texas Press, Austin.
- SWOFFORD, D.L. 2001. *PAUP\*: Phylogenetic analysis using parsimony (\* and other methods)*. Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.