Phylogenetics and biogeography of the microendemic rodent *Xerospermophilus perotensis* (Perote ground squirrel) in the Oriental Basin of Mexico

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Phylogenetic relationships of the Mexican endemic and endangered Perote ground squirrel, *Xerospermophilus perotensis* (Rodentia: Sciuridae), were examined using 2 mitochondrial (cytochrome-

\[\text{Cytb}\]

and 12S ribosomal RNA) and 2 nuclear (growth hormone receptor and interphotoreceptor retinoid-binding protein) genes for a total of 3,403 base pairs. Gene sequences were analyzed using maximum-likelihood and Bayesian models of phylogenetic inference. Independent analyses of the 4 gene sequences converged on essentially identical gene trees, all showing *X. perotensis* to be sister to *X. spilosoma* from San Luis Potosí, Mexico, and nested within other geographic samples of *X. spilosoma*. Given the current absence of diagnostic morphological characters to distinguish *X. perotensis* from *X. spilosoma* and the moderate level of *Cytb* sequence divergence between the 2 forms (3.6%, which is less than divergence values measured between other subspecies of *X. spilosoma*), *X. perotensis* is herein reduced to subspecies status as *X. spilosoma perotensis*. Based on molecular estimates of divergence times, phyletic diversification of the genus *Xerospermophilus* began near the end of the Pliocene and *X. spilosoma perotensis* diverged from other Mexican populations of *X. spilosoma* during Pleistocene times. Climate cycles during the Pleistocene and the final uplift of the Trans-Mexico Volcanic Belt may have played a major role in early diversification in this lineage.

Key words: biogeography, endemic species, Mexican Plateau, molecular differentiation, phylogenetic analyses, pygmy ground squirrel, Trans-Mexico Volcanic Belt

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The concept of endemism is central to the fields of biogeography and biological conservation, and areas with high numbers of endemic species, or “biodiversity hot spots,” often are included in protected area networks (Myers et al. 2000). In biogeography, an area of endemism usually is defined as a region that contains the only known occurrences of 2 or more taxa (i.e., multiple taxa are restricted to that region), but other definitions of “areas of endemism” focus on the area’s geographic delimitation by natural barriers or the distributional congruence of several species in the area (Harold and Mooi 1994; Hausdorf 1998; Lomolino et al. 2010; Platnick 1991).

Mexico is widely known as a biologically megadiverse country and a biodiversity hot spot (Lamoreux et al. 2006); the diverse Mexican biota is the product of interactions between a dynamic and complex topography and myriad ecological and historical factors (Velasco de León et al. 2007). In central Mexico, the highlands and arid valleys of the Trans-Mexico Volcanic Belt are home to one of the most diverse biotas in the world (Luna et al. 2007).

Among the mountains at the southeastern edge of the Trans-Mexico Volcanic Belt lies the Oriental Basin (Cuenca Oriental; Fig. 1). By any definition of “area of endemism,” this semiarid, endorheic (closed drainage) basin, which covers portions of the states of Puebla, Tlaxcala, and Veracruz, is an important area of endemism in North America. This relatively small (approximately 5,000-km²) basin is characterized by alkaline grasslands, bunch grasses, and aridland scrubs in the valleys and coniferous forests in the surrounding mountains (Valdéd and Ceballos 1997). The basin supports several endemic taxa...
of plants and animals, including at least 4 taxa of endemic mammals: the Oriental Basin pocket gopher (Cratogeomys fulvescens—Hafner et al. 2005), the Perote deermouse (Peromyscus bullatus—González-Ruiz and Álvarez-Castañeda 2005), Nelson’s woodrat (Neotoma nelsoni—González-Ruiz et al. 2006), and the Perote ground squirrel (Xerospermophilus perotensis—Best and Ceballos 1995). As noted by Hafner and Riddle (2005), the Oriental Basin has been subjected to extensive agricultural conversion, resulting in damage or loss of much of the native desert habitat.

Shreve (1942) referred to the Oriental Basin as the southernmost extension of the Chihuahuan Desert, and most mammals of the Oriental Basin, including X. perotensis, are arid-adapted species. Typically, the closest relatives of Oriental Basin endemics inhabit the deserts of the Mexican Plateau to the north, and this appears to be the case for X. perotensis, whose sister species is thought to be X. spilosoma (Fig. 1; Howell 1938).

In his revision of North American ground squirrels, Howell (1938) classified all ground squirrels in the genus Citellus (later transferred to Spermophilus by Hershkovitz [1949]) and divided the genus into 8 subgenera: Ammospermophilus, Callospermophilus, Citellus, Ictidomys, Notocitellus, Otospermophilus, Poliocrateus, and Spermophilus. Early morphological and chromosomal studies suggested a close relationship between Spermophilus perotensis and S. spilosoma (Howell 1938; Uribe-Alcocer and Ahumada-Medina 1990); however, composition of and relationships among the subgenera of Spermophilus were not clearly understood at that time. S. perotensis and S. spilosoma were placed as sister taxa in the subgenus Ictidomys, along with I. tridecemlineatus, I. mexicanus, and the more recently described I. parvidens (Helgen et al. 2009).

The taxonomic status and systematic affinities of S. perotensis were not investigated again until molecular studies by Harrison et al. (2003) and Herron et al. (2004) confirmed the close affinity of S. perotensis with S. spilosoma. In fact, both molecular studies (using the same specimens of S. spilosoma and based on the cytochrome-b [Cytb] gene) showed S. spilosoma to be paraphyletic with respect to S. perotensis, with S. s. pallescens from Mexico sister to S. perotensis, and S. s. marginatus from Kansas sister to the S. s. pallescens + S. perotensis clade. These same studies showed the S. perotensis + S. spilosoma clade (subgenus Ictidomys) to be sister to the S. mohavensis + S. tereticaudus clade (subgenus Xerospermophilus), with prairie dogs (Cynomys) sister to this group.

Helgen et al. (2009) combined new morphological data with the molecular evidence provided by Harrison et al. (2003) and Herron et al. (2004) to elevate the subgenus Xerospermophilus to full generic status. In Xerospermophilus, Helgen et al. (2009) included the 4 species of pygmy ground squirrels adapted to arid and semiarid conditions: X. mohavensis, X. tereticaudus, X. spilosoma, and X. perotensis. In view of the potential paraphyly of X. spilosoma (Harrison et al. 2003; Herron et al. 2004), Helgen et al. (2009:294) recommended future research into species-level boundaries in the spilosoma–perotensis complex and suggested that “X. perotensis may prove to be best classified as the southernmost subpopulation of X. spilosoma.”

Despite previous morphological and molecular studies of the systematic status of X. perotensis and allied taxa, several uncertainties remain with respect to the species status of X. perotensis, monophyly of X. spilosoma, and the timing of diversification events within the genus Xerospermophilus relative to major geological and climatic events. Each of these issues is explored in this analysis using newly acquired samples of X. perotensis, X. spilosoma, and other members of the genera Xerospermophilus and Ictidomys, and sequence evidence from multiple mitochondrial and nuclear genes.

**Materials and Methods**

**Sampling.**—Tissue samples of I. mexicanus (n = 1 individual), I. parvidens (n = 2), I. tridecemlineatus (n = 4), X. mohavensis (n = 2), X. perotensis (n = 4), X. spilosoma (n = 3), and X. tereticaudus (n = 2) were either collected in the field under the authority of Mexican collecting permit FAUT-0002 (issued to F. A. Cervantes) or donated by museums (Appendix I). In addition to the DNA sequences generated in this study, 34 sequences were downloaded from GenBank for use in the molecular analyses (Appendix I). Outgroups in the analyses included specimens of Urocitellus townsendii (in the Cytb analysis), Callospermophilus lateralis (in the 12S ribosomal
RNA [12S] and interphotoreceptor retinoid-binding protein [IRBP] analyses), and species of the genus Ictidomys (in the growth hormone receptor [GHR] analysis). The collection and processing of samples were undertaken following the guidelines of the American Society of Mammalogists for use of wild animals in research (Kelt et al. 2010; Sikes et al. 2011).

Laboratory protocols.—Total genomic DNA was extracted from tissue using a commercial kit (DNeasy Blood and Tissue Kit; Qiagen Inc., Valencia, California). Portions of 2 nuclear genes (GHR and IRBP), and 2 mitochondrial genes (Cybt and 12S) were sequenced for subsequent analysis. The genes were amplified by polymerase chain reaction (Saiki et al. 1988) using the following universal primers developed for rodents: 12S L82 and 12S H900 for 12S amplification of the following parameters: initial denaturation at 94°C for 5 min followed by 34 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 1 min, extension at 72°C for 1.5 min, and 1 final extension at 72°C for 10 min. Amplification of the IRBP gene began with initial denaturation at 95°C for 10 min followed by 27 cycles of denaturation at 95°C for 25 s, annealing at 58°C for 20 s, extension at 72°C for 1 min, and 1 final extension at 72°C for 10 min. Amplification of both mitochondrial genes began with initial denaturation at 95°C for 2 min followed by 27 cycles at 95°C for 1 min, annealing at 49°C for 1 min, extension at 72°C for 2 min, and 1 final extension at 72°C for 7 min (Mantooth et al. 2000). Amplifications were performed in a total volume of 25 μl and 200 ng of DNA. Agarose gels (2%) were used to visualize amplified products. Polymerase chain reaction products were purified using either polyethylene glycol or ExoSAP-IT (Affymetrix, Santa Clara, California). DNA sequencing was performed by the University of Missouri-Kansas City DNA Sequencing Core at the University of Missouri-Kansas City. DNA sequencing was conducted using the dye terminator cycle sequencing method. Following analyses of individual genes, a partitioned analysis including all 4 genes was conducted using ML and BI frameworks. ML analyses were run in both PAUP* and PhyML 3.0 (Guindon and Gascuel 2003). Maximum-likelihood analyses were performed with the starting trees obtained from 100 random, stepwise additions followed by tree-bisection-reconnection branch swapping. In the BI analyses, best-fit models were applied to each data partition with unlinked parameters and allowing rate variation. The Metropolis Markov chain Monte Carlo analysis consisted of 2 independent runs of 10 x 10^6 generations in which trees were sampled every 10^3 generations, resulting in 10^4 samples for each run. After discarding the initial 10% as burn-in, a majority-rule consensus tree was constructed using the final 18 x 10^3 trees. The analysis was stopped when the average standard deviation of split frequencies approached zero (<0.01) and convergence was reached, as determined using Tracer version 1.5 (Rambaut and Drummond 2007) and AWTY (Nylander et al. 2008). The combined data set was analyzed in a partitioned manner (genes and model parameters) to allow for independent convergence on optimal values for each component (Ronquist and Huelsenbeck 2003). Data partitions included mitochondrial versus nuclear genes, Cybt versus 12S, Cytb versus IRBP, and 12S versus IRBP genes. Nodes were considered well supported if there was >80% bs support in ML analyses or >95% pp in BI analyses.

Estimates of genetic divergence.—The identification of different rates of DNA substitution in a data set is not uncommon, and the use of a correction for the unobserved substitutions is suggested (Felsenstein 2004). To enable comparison of my results with those of previous studies (Harrison et al. 2003; Herron et al. 2004), sequence divergence values for the Cybt gene were corrected using the Kimura 2-parameter substitution model (Kimura 1980) in PAUP* 4.0b10 (Swofford 2003) and MEGA version 5 (Tamura et al. 2011). Saturation analyses for 3rd codon positions were performed using the methods of Griffiths (1997), and maximum-likelihood (ML) analyses were run with and without 3rd codon transitions to evaluate the effects of 3rd codon substitutions on phylogenetic reconstruction.

Phylogenetic analyses.—Initial phylogenetic analyses were conducted using Bayesian inference (BI) in the program MrBayes (version 3.2-cvs—Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and ML in PAUP*. Analysis of the Cytb data included 37 sequences of 14 species for which complete Cytb sequences were available. Phylogenetic analysis of the J2S gene included 17 specimens of 8 species, analysis of GHR included 14 specimens of 6 species, and analysis of IRBP included 16 specimens representing 8 species (Appendix I). Following analyses of individual genes, a partitioned analysis including all 4 genes was conducted using ML and BI frameworks. ML analyses were run in both PAUP* and PhyML 3.0. Best-fit models for ML and BI analyses were evaluated using the Akaike information criterion and the program jModeltest 0.1.1 (Guindon and Gascuel 2003; Posada 2008). The following models were selected for Cytb, J2S, GHR, and IRBP genes, respectively: TrN + G, TIM3 + G, TPTM3UF + I + G, and TPTM3UF + I. ML clade support was assessed using 500 bootstrap (bs) replicates in PAUP* and PhyML 3.0, and clade support in the BI analyses was evaluated using posterior probabilities (pp).

Maximum-likelihood analyses were performed with the starting trees obtained from 100 random, stepwise additions followed by tree-bisection-reconnection branch swapping. In the BI analyses, best-fit models were applied to each data partition with unlinked parameters and allowing rate variation. The Metropolis Markov chain Monte Carlo analysis consisted of 2 independent runs of 10 x 10^6 generations in which trees were sampled every 10^3 generations, resulting in 10^4 samples for each run. After discarding the initial 10% as burn-in, a majority-rule consensus tree was constructed using the final 18 x 10^3 trees. The analysis was stopped when the average standard deviation of split frequencies approached zero (<0.01) and convergence was reached, as determined using Tracer version 1.5 (Rambaut and Drummond 2007) and AWTY (Nylander et al. 2008). The combined data set was analyzed in a partitioned manner (genes and model parameters) to allow for independent convergence on optimal values for each component (Ronquist and Huelsenbeck 2003). Data partitions included mitochondrial versus nuclear genes, Cybt versus 12S, Cytb versus IRBP, and 12S versus IRBP genes. Nodes were considered well supported if there was >80% bs support in ML analyses or >95% pp in BI analyses.
dated fossil (Goodwin 1995; Harrison et al. 2003; Pizzimenti 1975) to constrain the minimum date for separation of *Cynomys* from *Xerospermophilus* to 2.7 million years ago (mya). To account for uncertainty in the fossil-based calibration, the fossil date was modeled on a lognormal distribution rather than a point calibration (Ho and Phillips 2009). The analysis used a relaxed clock with a lognormal distribution allowing rate variation among sites. Two independent analyses were run for $2 \times 10^7$ generations each, sampling the parameter every $10^3$ generations. Convergence statistics were checked for effective sample sizes using Tracer version 1.5 and AWTY. Consensus trees were generated from the resulting $20 \times 10^3$ trees using TreeAnnotator version 1.6.0 (Rambaut and Drummond 2009) after elimination of 10% as burn-in.

**RESULTS**

Analyses of DNA sequences involved a total of 3,403 base pairs (bp), including 1,141 bp of *Cytb*, 736 bp of *12S*, 910 bp of *GHR*, and 616 bp of *IRBP*. ML and BI analyses using only *Cytb* sequences from the 37 individuals with complete *Cytb* sequences recovered trees with identical branch structure (Fig. 2). In these trees, there is strong support ($pp = 1.00$; $bs = 100\%$) for monophyly of the genus *Xerospermophilus*. Within *Xerospermophilus*, *X. perotensis* is nested within *X. spilosoma*, and most closely allied with its geographically closest neighbor in the state of San Luis Potosí on the Mexican Plateau (locality 3 in Fig. 1). The other samples of *X. spilosoma* from Durango (locality 6), Kansas (locality 5), and New Mexico (locality 4) are added to the tree in a stepwise fashion. *X. mohavensis* and *X. tereticaudus* also are depicted as sister taxa. Among the many outgroups used in the analysis (listed above and in Appendix I), the genus *Cynomys* was found to be sister to *Xerospermophilus*, although this relationship was not well supported ($pp = 0.84$; $bs = 79\%$) and therefore is not shown in Fig. 2.

The *Cytb* divergence values (Table 1) show the taxa included in Fig. 2 to be well differentiated genetically. *X. perotensis* is 3.6% genetically divergent from the *X. spilosoma* sample from San Luis Potosí, and these 2 populations together show an average *Cytb* divergence of 6.4% from the other samples of *X. spilosoma* from Durango, Kansas, and New Mexico. The *X. perotensis* + *X. spilosoma* clade (including all samples of *spilosoma*) shows an average of 11.2% sequence divergence from the *X. mohavensis* + *X. tereticaudus* clade and 11.8% divergence from specimens of the genus *Cynomys*.

Independent BI and ML analyses of *12S*, *GHR*, and *IRBP* sequences confirmed the *Cytb* topology shown in Fig. 2, although nodal support values varied widely depending on the gene analyzed (trees not shown but available on request). In all analyses, the genus *Xerospermophilus* was depicted as monophyletic and sister to *Cynomys*, and the sister species status of *X. mohavensis* + *X. tereticaudus* was confirmed with strong support. All the samples of *X. spilosoma* and *X. perotensis*...
formed a monophyletic group, and the sister relationship between X. perotensis and the X. spilosoma sample from San Luis Potosí was recovered, but with low nodal support in the analyses of the nuclear genes (GHR and IRBP).

The BI and ML analyses of the partitioned data sets (partitioned by mitochondrial genes only, nuclear genes only, and mitochondrial + nuclear genes) focused on relationships within Xerospermophilus (Fig. 3). In all partitioned analyses, the genus Xerospermophilus was depicted as monophyletic with high support values. Again, the samples of X. spilosoma and X. perotensis formed a monophyletic group, and the sister status of X. perotensis and X. spilosoma from San Luis Potosí was confirmed with high nodal support (pp > 0.97; bs = 100%).

Mean estimates of divergence times (Fig. 2) ranged from a low of 0.7 mya between X. perotensis and X. spilosoma (San Luis Potosí) to a high of 3.5 mya between the genera Cynomys and Xerospermophilus. All estimates of divergence times within the genus Xerospermophilus place these events in the Pleistocene, with the possible exception of the split between the X. mohavensis + X. tereticaudus clade and the X. perotensis + X. spilosoma clade, which was estimated at 2.7 mya with a confidence interval extending from 4.3 to 1.3 mya (Fig. 2).

**DISCUSSION**

This study of mitochondrial and nuclear DNA sequences confirms that X. perotensis is a genetically well-differentiated unit within Xerospermophilus. The sister relationship between X. perotensis and the sample of X. spilosoma from San Luis Potosí (representing the subspecies X. s. cabrerai) also is strongly supported in this study and is consistent with evidence provided by Uribe-Alcocer and Ahumada-Medina (1990), who reported chromosomal similarities between X. perotensis and X. s. cabrerai and interpreted this as an indicator of a close phylogenetic relationship between these taxa.

**Species status of X. perotensis.**—Since its original description by Merriam (1893), X. perotensis has been considered a valid species. However, recent morphological and molecular studies have questioned its species status, and some authors have suggested that X. perotensis is best regarded as a subspecies of X. spilosoma (Harrison et al. 2003; Helgen et al. 2009; Herron et al. 2004). The present study confirms that continued recognition of X. perotensis at the species level renders X. spilosoma paraphyletic (Figs. 2 and 3). Paraphyly of X. spilosoma could be resolved taxonomically by recognizing multiple species within X. spilosoma, but unless one recognizes species based solely on degree of genetic divergence, no evidence is available at this time suggesting that X. spilosoma is a composite of multiple cryptic species. It also could be argued that populations of X. perotensis and X. spilosoma from San Luis Potosí (X. s. cabrerai) should be combined into a single species. However, again, there is no evidence for species-level divergence between X. s. cabrerai and other subspecies of X. spilosoma except for the relatively large Cytb distances measured between the subspecies examined in this study (5.2–7.9%; Table 1). Synonymization of X. s. cabrerai with X. perotensis still would require recognition of multiple species within X. spilosoma to maintain monophyletic taxa.

Sister species within the sciurid genera Cynomys and Marmota show Cytb divergence values ranging from 1.2% to 7.7% (Harrison et al. 2003; Steppan et al. 1999), so the divergence value calculated between X. perotensis and X. s.
take the conservative route and recognize *X. perotensis* as a subspecies of rodents. For example, *X. perotensis* resembles *X. s. pallescens* of the northern Mexican Plateau and Sierra Madre Oriental, except that *X. perotensis* is larger overall, has a shorter tail, is more yellowish dorsally, and has smaller and less-conspicuous buffy spots (Best and Ceballos 1995). The skull of *X. perotensis* is similar to that of *X. s. spilosoma* (found in southern Durango, Zacatecas, and parts of nearby states), except that the skull of *X. perotensis* is larger, has a relatively narrower and higher braincase, has auditory bullae that are broader and more flattened, and has molariform teeth that are heavier than those of *X. s. spilosoma* (Best and Ceballos 1995; Helgen et al. 2009; Uribe-Alcocer et al. 1978).

Considering the absence of morphological or chromosomal evidence supporting the species status of *X. perotensis*, I herein take the conservative route and recognize *perotensis* as a subspecies of *X. spilosoma* (as *X. s. perotensis*). The relatively high divergence values measured between the subspecies of *X. spilosoma* in this study (Table 1) may signal presence of multiple cryptic species, but if so, recognition of additional species must await a thorough study of geographic variation throughout the range of *X. spilosoma*.

**Biogeography of Xerospermophilus on the Mexican Plateau and in the Oriental Basin.**—Shreve (1942) recognized that the Mexican Plateau, the Oriental Basin, and other isolated arid and semiarid regions of central Mexico were relicts of a once-continuous southern extension of the Chihuahuan Desert. More recent research suggests that the arid and semiarid lands of central Mexico were continuous until the mid- to late Miocene, when rise of the Trans-Mexico Volcanic Belt began to act as a barrier between populations of arid-adapted species (Ferrusquía-Villafranca and González 2005; Ferrusquía-Villafranca et al. 2005). Hoffmann and Jones (1970) examined present-day distributions of several mammal species in Mexico and suggested that many prairie and desert species may have reached their southernmost distributions during Pleistocene times, with subsequent range contractions leaving isolated populations in the south. They suggested that *Cynomys mexicanus* was one such peripheral isolate of the once more-widespread species, *C. ludovicianus* (Hoffmann and Jones 1970). The Perote ground squirrel, *X. s. perotensis*, isolated in the Oriental Basin of central Mexico, may be another example of this phenomenon.

Uribe-Alcocer and Ahumada-Medina (1990) speculated that *X. spilosoma* stock was once widespread throughout the highlands of northern Mexico and was able to disperse southward because of continuous, dry habitats found in intermontane valleys. Subsequent tectonic or climatic events, or a combination of both, during the Pleistocene fragmented the once-continuous grassland habitat in central Mexico, leaving the present-day patches of arid and semiarid habitats, including the Oriental Basin (Ferrusquía-Villafranca and González 2005; Ferrusquía-Villafranca et al. 2005; Hoffmann and Jones 1970; Pizzimenti 1975).

The results of this study underscore the biotic importance of 3 understudied biogeographic regions of Mexico: the Oriental Basin (inhabited by *X. s. perotensis*), the Mexican Plateau (*X. s. cabrerai*), and the Bolsón de Mapimi (*X. s. pallescens*). The origin of the Oriental Basin is closely linked with the volcanic activity that gave rise to alkaline lakes and the rain-shadow effect that caused isolated pockets of arid and semiarid land in the Trans-Mexico Volcanic Belt (Caballero et al. 2003; Morán-Zenteno 1994). The Mexican Plateau formed as a result of uplift of the Sierra Madre Oriental, Sierra Madre Occidental, and Trans-Mexico Volcanic Belt, which created a dry tableland in the rain shadow of these large mountain ranges. Recent phylogenetic and biogeographic studies are beginning to show the importance of the Mexican Plateau as a center of evolutionary divergence in many rodent taxa (Fernández et al. 2012; Neiswenter and Riddle 2010). Finally, the Bolsón de Mapimi, a closed desert basin located north of the Sierra de San Luis Potosí, Sierra de Zacatecas, and Sierra de la Breña, formed during the Wisconsinan glacial period of the Pleistocene and acted as a refugium for many desert organisms (Elias 1992), including the ancestors of *X. s. pallescens*.

**Resumen**

Se examinaron las relaciones filogenéticas de la ardilla terrestre de Perote *Xerospermophilus perotensis* (Rodentia: Sciuridae), especie mexicana endémica y amenazada, usando 2 genes mitocondriales (Citromoco b [Citb] y 12S ARN ribosomal) y 2 genes nucleares (Receptor de la hormona del crecimiento y la Proteína de unión al retinoide intersticial) para un total de 3,403 pares de bases. Las secuencias genéticas fueron analizadas usando máxima verosimilitud y modelos Bayesinos de inferencia filogenética. Los análisis independientes de las secuencias de los 4 genes convergieron esencialmente en árboles de genes idénticos, todos mostrando a *X. perotensis* como taxa idénticos de *X. spilosoma* de San Luis Potosí, México y anidados dentro de otras muestras geográficas de *X. spilosoma*. Dada la ausencia actual de caracteres morfológicos diagnósticos para distinguir *X. perotensis* de *X. spilosoma* y el nivel moderado de divergencia de las secuencias de *Citb* entre las 2 formas (3.6%, que es menor a los valores de divergencia medidos entre otras subespecies de *X. spilosoma*), *X. perotensis* aquí se reduce a status subespecífico como *X. spilosoma perotensis*. Basado en estimaciones moleculares de tiempo de divergencia, la diversificación filética del género *Xerospermophilus* comenzó cerca del final del Plioceno y *X. spilosoma perotensis* divergió de otras poblaciones mexicanas de *X. spilosoma* durante el Pleistoceno. Los ciclos climáticos durante el Pleistoceno y el alzamiento final del Eje Neovolcánico Trans-Mexicano pudieron jugar un papel importante en la diversificación temprana de este linaje.

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**Literature Cited**


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**APPENDIX I**

Specimens used in the analysis of phylogenetic relationships of Xerospermophilus spilosoma perotensis. Specimens are listed alphabetically by taxon and locality; geographic coordinates, elevation, catalogue number, sequenced genes, and GenBank numbers are provided. Specimens are housed in the following museums: Colección Nacional de Mammíferos, Instituto de Biología, Universidad Nacional Autónoma de México (CNMA); Cornell University DNA collection (CU; samples from CU are followed by the collector’s field number in parentheses); Cornell University DNA Sample Number (S); Los Angeles County Museum of Natural History (LACM); Louisiana State University Museum of Natural Science (LSUMZ); Colección de Mammíferos del Museo de Zoológia “Alfonso L. Herrera,” Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC); New Mexico Museum of Natural History (NMNH); and the Museum of Southwestern Biology, University of New Mexico (MSB). Numbers in parentheses in boldface type before localities indicate localities for specimens of Xerospermophilus mapped in Fig. 1.

Callospermophilus lateralis.—United States: California, Mono Co., Sweetwater Mountains; Sweetwater Canyon, Nugent Cabin, 38.469445, −119.265487, 2,100 m, LACM 85487, L25 = AY227530, IRBP = AY227586.

Cynomys gunnisoni.—United States: Arizona, Apache Co., Petrified Forest National Park, 34.909, −109.806, 1,641 m, S 75 (73), Cytb = AF157923; CU 82 (WA1), Cytb = AF157930.
Cynomys leucurus.—United States: Utah, Uintah Co., 8 km E Jensen on Highway 40, CU 1 (EY 1138), 40.369, –109.240, 1.689 m, Cytb = AF157838; 12S = JX027528, IRBP = JX027584. Utah, Uintah Co., 11 km NW Bonanza on Highway 45, CU 2 (EY1137), 40.094, –109.269, 1.572 m, Cytb = AF157879.

Cynomys ludovicianus.—United States: Nebraska, Omaha Zoo CU 38 (1A), Cytb = AF157890; CU 41 (4A), Cytb = AF157892; collection locality not available, CU 1 (EY 1138), IRBP = JX027584.

Cynomys mexicanus.—Mexico: Nuevo León, Ejido El Tokio, 18 km E Highway 57 on Highway 58, 24.6, –100.2, 1.891 m, CU 101 (EY1180), Cytb = AF157841; CU 102 (EY1181), Cytb = AF157842.

Cynomys parvidens.—United States: Utah, Kane Co., Bryce Canyon National Park, 1 km from Visitor Center, 37.58, –112.18, 2.466 m, CU 74 (BCU 1), Cytb = AF157922; Utah, Kane Co., Bryce Canyon National Park, 20 km from boundary, 37.58, –112.18, 2.466 m, CU 81 (BC1), Cytb = AF157929.

Cynomys mexicanus mexicanus.—Mexico: México, Parque Nacional Zoquiapan, 15 km SW Rio Frío, 19.3, –98.6, 2.980 m, CU 108 (EY 1210), Cytb = AF157848.

Cynomys parvus.—United States: New Mexico, Chaves Co., Eastern New Mexico University, Roswell, West Wells and University Street, 33.39, –104.52, 1.977 m, MSB 135244, Cytb = JX047304, 12S = JX047294, GHR = JX047262; Mexico: Nuevo León, 3 km NE Apodaca, approximately 23 km NE Monterrey, 25.80, –100.16, 4.08 m, CU 111 (EY 1197), Cytb = AF157852; CU 112 (MVA 105), Cytb = AF157853.

Cynomys tridecemlineatus.—United States: Kansas, Finney Co., Garden City, 37.9, –100.8, 8.66 m, CU 13 (EY 1147), Cytb = AF157870; CU 14 (EY 1148), Cytb = AF157877; collection locality not available (from personal collection of R. L. Honeycutt), H 2147, 12S = U67290, IRBP = AF287278; South Dakota, Union Co., 1 mile N, 1.5 miles W Junction City, 42.800, –96.815, 377 m, LSUMZ 10692, Cytb = JX047305, 12S = JX047295, GHR = JX047260; South Dakota, Clay Co., 3.5 miles N, 3.5 miles W Vermillion, 42.829, –96.857, 376 m, LSUMZ 10728, Cytb = JX047306, 12S = JX047296, GHR = JX047261, IRBP = JX047282.

Xerocomellus mohavensis.—United States: (1) California, San Bernardino Co., 9 miles NNE Johannesburg, 35.473, –117.589, 1.075 m, MSB 40496, 12S = JX047290, GHR = JX047273, IRBP = JX065593; MSB 40503, Cytb = JX047298, 12S = JX047291, GHR = JX047272, IRBP = JX047274.

Xerocomus spilosoma perotensis.—Mexico: (2) Puebla, Tepeyahualco, 19.490, –97.489, 2.336 m, CNMA 37253, Cytb = AF157948, 12S = JX047289, GHR = JX047271, IRBP = JX047277; CNMA 37254, Cytb = AF157840, JX047302, 12S = JX047286, GHR = JX047268, IRBP = JX047279; Veracruz: Municipality of Perote, 3 km S El Frijol Colorado, 19.572, 97.383, 2.435 m, MZFC 11089, Cytb = JX047303, 12S = JX047287, GHR = JX047269, IRBP = JX047278; Municipality of Perote, 5 km W Perote, 19.587, –97.330, 2.400 m, MZFC 11090, Cytb = JX047301, 12S = JX047288, GHR = JX047270, IRBP = JX065593.

Xerocomus spilosoma caborrensis.—Mexico: (3) San Luis Potosí, 10 miles S Villa de Ramos, 22.666, –101.953, 2.200 m, NNMMNH 3651, Cytb = JX047299, 12S = JX047283, GHR = JX047263, IRBP = JX065594.

Xerocomus spilosoma spilosoma marginatus.—United States: (4) New Mexico, Bernalillo Co., 8 km W Albuquerque, 35.084, –106.738, 1.631 m, LSUMZ 01, 12S = JX047285, GHR = JX047264, IRBP = JX047276; LSUMZ 05, Cytb = JX047300, 12S = JX047284, GHR = JX047265, IRBP = JX047275. (5) Kansas, Finney Co., 13 km S, 2 km E Holcomb, 37.872, –100.964, 893 m, CU 3 (EY1142), Cytb = AF157885; CU 6 (EY1146), Cytb = AF157911.

Xerocomellus spilosoma pallescens.—Mexico: (6) Durango, 4 km E Ceballos, 26.523, –104.089, 1.117 m, CU 105 (EY 1195), Cytb = AF157845; Durango, Ejido La Flor, 20 km E Ceballos, 26.524, –103.929, 1.146 m, CU 106 (EY 1193), Cytb = AF157846.

Xerocomus spiculosus terecuta basiliscus.—United States: (7) Arizona, Pima Co., Tucson, 32.221, –110.926, 917 m, MSB 86022, 12S = JX047292, GHR = JX047266, IRBP = JX047280; MSB 92638, Cytb = JX047297, 12S = JX047293, GHR = JX047267, IRBP = JX047281; Arizona, Pima Co., 18 km W Tucson, Ryan Field, 32.223, –111.116, 735 m, CU 91 (EY 1169), Cytb = AF157940; CU 92 (EY 1167), Cytb = AF157941.

Polioctellus franklinii.—United States: Nebraska, Omaha Zoo CU 42 (1A), Cytb = AF157893; CU 43 (2A), Cytb = AF157894.

Urocitellus townsendii idahoensis.—Collection locality not available, CU 86 (EY 1062), Cytb = AF157949.

Urocitellus townsendii.—Collection locality not available, CU 87 (EY 1064), Cytb = AF157938.