COMPARATIVE BIOGEOGRAPHY OF THE ARID LANDS OF CENTRAL MEXICO

A Dissertation

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DEDICATION

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TABLE OF CONTENTS

DEDICATION .................................................................................................................................................. ii

ACKNOWLEDGEMENTS ................................................................................................................................ iii

LIST OF TABLES ............................................................................................................................................ vii

LIST OF FIGURES ........................................................................................................................................ viii

ABSTRACT .................................................................................................................................................... x

CHAPTER 1. INTRODUCTION .................................................................................................................. 1
  1.1 Literature Cited .................................................................................................................................. 10

CHAPTER 2. MOLECULAR PHYLOGENETICS AND BIOGEOGRAPHY OF A RARE ENDEMIC: NELSON’S WOODRAT (NEOTOMA LEUCODON NELSONI) IN THE ORIENTAL BASIN OF MEXICO.
  2.1 Introduction ..................................................................................................................................... 16
  2.2 Materials and Methods .................................................................................................................... 19
  2.3 Results ............................................................................................................................................ 24
  2.4 Discussion ....................................................................................................................................... 29
  2.5 Literature Cited ................................................................................................................................ 36

CHAPTER 3. PHYLOGENETICS AND BIOGEOGRAPHY OF THE MICROENDEMIC RODENT XEROSPERMOPHILUS PEROTENSIS (PEROTE GROUND SQUIRREL) IN THE ORIENTAL BASIN OF MEXICO.
  3.1 Introduction..................................................................................................................................... 43
  3.2 Materials and Methods .................................................................................................................... 47
  3.3 Results ............................................................................................................................................ 51
  3.4 Discussion ....................................................................................................................................... 56
  3.5 Literature Cited ................................................................................................................................ 59

  4.1 Introduction ..................................................................................................................................... 64
  4.2 Materials and Methods .................................................................................................................... 67
  4.3 Results ............................................................................................................................................ 71
  4.4 Discussion ....................................................................................................................................... 74
  4.5 Literature Cited ................................................................................................................................ 81

CHAPTER 5. MOLECULAR SYSTEMATICS AND BIOGEOGRAPHY OF THE MEXICAN ENDEMIC KANGAROO RAT, DIPodomys phillipsii
LIST OF TABLES

Table 2.1.—Cytochrome-\textit{b} sequence divergence values (Kimura 2-parameter model) between and within species of the \textit{Neotoma micropus} and \textit{N. floridana} species group ................................................................. 26

Table 3.1.—Mean percent cytochrome-\textit{b} sequence divergence values (Kimura 2-parameter model) among species of the genus \textit{Xerospermophilus} and \textit{Cynomys} ........................................................................................................ 53

Table 4.1.—Percent sequence divergence in the cytochrome-\textit{b} gene (Kimura 2-parameter model) among 8 taxa of \textit{Peromyscus}, including the 5 currently recognized subspecies of \textit{P. difficilis} ........................................................................................................ 72

Table 5.1.—Mean percent cytochrome-\textit{b} sequence divergence values (Kimura 2-parameter model) and ranges (in parentheses) between and within the 2 major clades of \textit{Dipodomys phillipsii}, \textit{D. elator}, and \textit{D. merriami} ......................................................... 101

Table 6.1.—Overview of taxonomic conclusion, divergence values between clades of interest, and mean estimates of divergence time for clades of interest (in million years) ................................................................................. 121
LIST OF FIGURES

Fig. 1.1.—Map of Mexico showing main geographic features discussed in this dissertation ........................................................................................................................................................................ 3

Fig. 1.2.—Map of Central Mexico showing the Oriental Basin and surrounding volcanoes ........................................................................................................................................................................ 5

Fig. 2.1.—Geographical distribution of the Neotoma micropus species group ............17

Fig. 2.2.—Map of central Mexico showing the known geographic distribution of Nelson’s woodrat, Neotoma leucodon nelsoni) .................................................. 20

Fig. 2.3.—Cladogram showing the placement of Nelson’s woodrat, N. leucodon nelsoni, within the genus Neotoma ................................................................. 27

Fig. 2.4.—Cladogram showing placement of Nelson’s woodrat, N. leucodon nelsoni, within the genus Neotoma ................................................................. 28

Fig. 2.5.—Phylogeny and chronogram of selected species of the genus Neotoma (and outgroups), and estimated divergence times calculated from *BEAST analysis .................................................................................................................................................. 30

Fig. 3.1.—Geographical distribution of pygmy ground squirrels of the genus Xerospermophilus ........................................................................................................ 44

Fig. 3.2.—Relationships among major clades of Xerospermophilus based on maximum likelihood and Bayesian analyses of Cytochrome-b sequences .................. 52

Fig. 3.3.—Relationships among major clades of Xerospermophilus based on a maximum likelihood analysis of Cytb, 12S, GHR, and IRBP sequences ........... 55

Fig. 4.1.—Geographical distribution of Peromyscus difficilis and P. nasutus in Mexico and the United States ........................................................................................................ 65

Fig. 4.2.—Phylogenetic relationships among the 5 subspecies of P. difficilis, P. nasutus, and allied taxa based on a maximum likelihood (ML) analysis of cytochrome-b sequences .................................................................................................................. 73

Fig. 4.3.—Phylogenetic relationships among selected specimens of P. difficilis and P. nasutus based on a partitioned maximum likelihood (ML) analysis of 2 mitochondrial genes and 2 nuclear genes .................................................................................................................. 75

Fig. 4.4.—Estimates of divergence times in P. difficilis, P. felipensis, and close relatives calculated from *BEAST analysis ........................................................................... 78
Fig. 5.1.—Map of central Mexico showing the geographic distribution of Phillips’ kangaroo rat, *Dipodomys phillipsii* ................................................................. 89

Fig. 5.2.—Phylogram showing placement of Phillips’ kangaroo rat, *D. phillipsii*, within the genus *Dipodomys* ........................................................................... 98

Fig. 5.3.—Phylogram showing placement of Phillips’ kangaroo rat, *D. phillipsii*, within allied species of *Dipodomys* ................................................................. 100
Most biogeographic studies on the Mexican biota have assumed that the dramatic climate cycles of the Pleistocene epoch and the prominence of the Trans-Mexico Volcanic Belt (TMVB) have played major roles in the origin and diversification of species. Here I studied the pylogenetics and biogeography of four codistributed rodent species. In each case, I have generated a phylogenetic hypothesis for the taxon and allied species using two mitochondrial (Cytochrome-b and 12S), and two nuclear genes (GHR and IRBP), I recommended appropriate taxonomic changes, and generated a temporal framework to identify events that may have produced the phylogenetic pattern.

Nelson’s woodrat *Neotoma nelsoni* and the Perote ground squirrel *Xerospermophilus perotensis*, were confirmed as having their closest relatives in the Mexican Plateau. My findings also confirmed that *N. nelsoni* and *X. perotensis* are genetically well-differentiated from their sister taxa. Genetic distances in combination with low levels of morphological differentiation suggest that they should be recognized only at the subspecific level as *N. leucodon nelsoni* and *X. spilosoma perotensis*. Molecular estimates of divergence times suggested that *N. l. nelsoni* and *X. s. perotensis* diverged from their sister taxa to the north during early Pleistocene times.

The rock mouse *Peromyscus difficilis* was divided into two well-supported clades, a northern clade including the subspecies *P. d. difficilis* and *P. d. petricola*, and a southern clade containing the subspecies *amplus, felipensis*, and *saxicola*. Molecular-based estimates of divergence times suggested that separation of these clades occurred in the Pleistocene.
My study of the Phillips’ kangaroo rat, *Dipodomys phillipsii*, revealed a biogeographic pattern different from that seen for other taxa. *D. phillipsii* was divided into two well-supported clades: one distributed on the Mexican Plateau, and a southern clade in the TMVB. Several lines of evidence supported my decision to return the Mexican Plateau clade of *D. phillipsii* to full species status as *D. ornatus*. My study showed that *D. phillipsii*, *D. ornatus*, *D. elator*, and *D. merriami* form a well-supported clade of kangaroo rats, but I was unable to resolve relationships among these four species. My molecular-based analyses of divergence times suggests that *D. phillipsii*, *D. ornatus*, *D. elator*, and *D. merriami* diverged in mid-Pliocene times, probably in or near the Mexican Plateau. Unlike the Pleistocene divergence dates reported in previous chapters this Pliocene divergence suggests that the morphotectonic processes that gave rise to the Trans-Mexico Volcanic Belt may have influenced early diversification in Mexican species of *Dipodomys*. 
CHAPTER 1
INTRODUCTION

Some of the oldest questions in biology focus on the origin of biological diversity. How do new species evolve? How do geography, geology, and climate influence the rate and timing of biological diversification? Do geographic barriers and environmental fluctuations affect all organisms equally? One of the disciplines that addresses these kinds of questions is biogeography. Biogeography is the study of the causes of and limitations on the geographic distribution of organisms through space and time (Nelson and Platnick 1981).

From a classical point of view, biogeography has been split into the subfields of historical biogeography and ecological biogeography. Historical biogeographers study the effect of past geographic phenomena on the distributional patterns and diversification of species, usually at or above the species level. In contrast, ecological biogeographers focus on the effects of climate fluctuations on the early diversification (incipient speciation) of populations, emphasizing demographic parameters at and below the species level (Nelson and Platnick 1981).

Populations of a species are not distributed evenly in space and time, and their distributional ranges can expand or contract directly or indirectly by environmental fluctuations, geological events, and other changes in a dynamic landscape. Through long periods of time, those changes may result in disjunct populations, and the combined affects of natural selection and genetic drift may result in differentiation between these isolated populations and their source populations. When differentiated populations of multiple species are exclusive to a particular geographic area, that area is known as an
area of endemism. Other definitions of “areas of endemism” focus on the area’s geographic delimitation by natural barriers or the distributional congruence of several species (Harold and Mooi 1994; Hausdorf 2002; Platnick 1991). The concept of endemism is central to biogeography and biological conservation and areas with high numbers of endemic species, or “biodiversity hotspots,” commonly are included in protected area networks (Myers et al. 2000).

México is widely known as a biologically megadiverse country and a biodiversity hotspot (Lamoreaux et al. 2006). The diverse Mexican biota is the historical product of complex interactions between an ever-changing topography and myriad ecological and environmental factors (Velasco de León et al. 2007). In central Mexico, the Mexican Plateau and the highlands and arid valleys of the Trans-México Volcanic Belt (TMVB) are home to one of the most diverse biotas in the world (Fig. 1.1; Cartron et al. 2005; Luna et al. 2007).

The Mexican Plateau is bounded by the Sierra Madre Occidental to the west, the Sierra Madre Oriental to the east, the Chihuahuan desert and the Sierra Zacatecas to the north, and the TMVB to the south (Fig. 1.1). It has the form of a parallelogram covering 85,300 km², with elevations ranging from 1,000 to 3,300 m. The climate varies from arid and hot to semiarid and temperate, and the vegetation is similar to that of nearby deserts, with xeric shrublands in the plains and pine-oak forests in the surrounding mountains (Ferrusquia-Villafranca et al. 2005). Recent studies of rodent populations in the Mexican Plateau have revealed complex patterns of diversification and endemism (Fernández et al. 2012; Neiswenter and Riddle 2010).
Fig. 1.1.—Map of Mexico showing main geographic features discussed in this dissertation (modified from a map produced by CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad; http://www.conabio.gob.mx/).
Among the mountains at the southeastern edge of the TMVB lies the Oriental Basin (Cuenca Oriental; Fig. 1.2). 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 for arid-adapted organisms in North America. This small (ca. 5,000 km²) basin characterized by alkaline grasslands, bunch grasses, and aridland scrubs in the valleys and coniferous forests in the surrounding mountains (Valdés and Ceballos 1997), is thought to be the southernmost extension of the Chihuahuan desert (Shreve 1942). The Oriental Basin supports several endemic relict taxa of plants and animals, including at least four endemic mammals: the Oriental Basin pocket gopher (Cratogeomys fulvescens), Nelson’s woodrat (N. nelsoni), the Perote deer mouse (Peromyscus bullatus), and the Perote ground squirrel (Xerospermophilus perotensis; Best and Ceballos 1995; González-Ruíz and Álvarez-Castaneda 2005; González-Ruíz et al. 2006; Hafner et al. 2005).

Past studies of biogeography and areas of endemism in Mexico have used a wide variety of approaches. Some researchers have made biogeographical inferences based on examination of distributional records analyzed with clustering algorithms, whereas others have used geographic information system (GIS) algorithms, often augmented by phylogenetic and phylogeographic information. These latter approaches allow the researcher to infer evolutionary relationships, estimate current and potential distributions, and model the potential effects of climatic and geological changes on the generation of diversity (Escalante et al. 2002, 2004; Marshall and Liebherr 2000).
Fig. 1.2—Map of Central Mexico showing the Oriental Basin and surrounding volcanoes: A) Iztaccíhuatl; B) Popocatépetl; C) Malinche; D) Pico de Orizaba; E) Cofre de Perote (modified from CONABIO maps). The Oriental Basin straddles the Mexican states of Puebla, Tlaxcala, and Veracruz.
Distribution-based studies.—Over the past 20 years, many studies of the Mexican flora and fauna have used panbiogeographic (or track) methodology. Panbiogeography emphasizes the spatial or geographic dimension of biodiversity and often is used in combination with Parsimony Analyses of Endemicity (PAE) and GIS (Balleza et al 2005; Escalante et al 2002, 2005; Luna et al 2004). Panbiogeography is a phenetic approach that uses knowledge of animal and plant distributions to draw inferences about the patterns and processes responsible for centers of diversity or areas of endemism (Craw et al. 1999). Many Mexican organisms have been studied using this approach, including aquatic organisms, insects, plants, mammals, and a combination of taxa from different groups (Andrés-Hernández et al. 2006; Corona and Morrone 2005; Corona et al. 2007; Escalante et al. 2003, 2004, 2007; Huidrobo et al. 2006; Katinas et al. 2004; Morrone 2004; Morrone and Escalante 2002; Morrone and Gutierrez 2005; Morrone and Marquez 2001; Torres and Luna 2006).

Cladistic biogeography.—Many researchers have studied the biogeography of Mexican organisms using cladistic biogeographic methods. These methods first reconstructs the phylogenetic relationships of multiple sets of taxa, then use this information to infer the relationships among the areas they occupy. Cladistic biogeography often is used in combination with Brooks Parsimony Analysis (BPA), an approach that has been used to study fishes, birds, or combinations of several taxa (Dominguez et al. 2006; Marshall and Liebherr 2000; Zink et al. 2000).

Phylogenetic studies.—Most systematic studies of Mexican taxa attempt to relate the phylogenetic patterns revealed in the study to current or past geographic barriers and climate. The major findings of this approach include discovery of geographic clades

Phylogeographic studies.—Studies relating the genetic architecture and spatial distribution of conspecific populations or closely related species are increasingly frequent in the literature (Avise 2009). Most phylogeographic studies describe population parameters, haplotype distributions related to geographic distance, patterns of expansion, retraction, and migration among populations, but also study species limits, timing of diversification, and the impact of vicariance and dispersal on phylogeographic patterns. Examples of the phylogeographic approach include many studies of vertebrate organisms, including fishes and amphibians (e.g., Doadrio and Domínguez 2004; Mulcahy and Mendelson 2000).

Comparative biogeography and phylogeography.—This relatively new approach, which involves the study of codistributed taxa, has been applied to few Mexican species to date. The main objective of this approach is to explore the effects of climate and geology on codistributed taxa to reveal common patterns and processes that may explain a shared evolutionary history (Avise 2000; Arbogast and Kenagy 2001). This approach has been used in studies of birds, fishes, mammals, and a mixed group of species in the TMVB and in the Chihuahuan, Sonoran, and Peninsular deserts of Mexico (Marshall and Lienherr 2000; Mateos 2005; Riddle and Hafner 2004; Zink 2002; Zink et al. 2000). This approach also has been implemented in conjunction with approximate Bayesian computation in studies of birds and pitvipers and colubrid snakes in the southern Mexican
highlands, the Peninsular Desert, and the Isthmus of Tehuantepec (Barber and Klicka 2010; Castoe et al. 2009; Daza et al. 2010; Leache et al. 2007).

My dissertation research uses the comparative phylogeographic approach to determine if a set of codistributed mammalian taxa show the same phylogenetic patterns and, if so, whether these patterns are the result from the same climatic and geologic events. Same topologies not necessarily imply common history for a set of codistributed taxa because geographic barriers may produce pseudocongruence in the form of soft and hard allopatric distributions. The first one implying an organismal response to environmental variations, the second implies external factors limiting distributions. (Pyron and Burbrink 2010). Well-supported phylogenetic inferences coupled with robust estimates of divergence times in codistributed species should be a powerful analytical tool to reveal underlying causes of lineage diversification (Bermingham and Moritz 1998; Hickerson et al. 2006).

Several arid-adapted rodents, including Nelson’s woodrat (*Neotoma nelsoni*), the Perote ground squirrel (*Xerospermophilus perotensis*), the rock mouse (*Peromyscus difficilis*), and Phillips’ kangaroo rat (*Dipodomys phillipsii*), show almost identical distributions in the Mexican Plateau and Oriental Basin of central Mexico. Not only are these species codistributed in their general ecological requirements, but their subspecific taxonomic boundaries are also very similar, and these boundaries are thought to result from effects of the TMVB. My study will test the hypothesis that the timing of major divergence events in the four rodent species listed above is coincident with the timing of major topographical shifts in the TMVB.

*Neotoma nelsoni* is a member of the rodent family Cricetidae (New World rats and
mice) and *Xerospermophilus perotensis* is a member of the Sciuridae (squirrels, marmots, and their relatives). Both species are endemic to the Oriental Basin and both are listed as threatened by the Mexican government. The putative sister species of both taxa are found in the Mexican Plateau to the north. Both species show slight, qualitative morphological differences when compared to their sister species, but scarcity of museum specimens and collecting restrictions by the Mexican Government have, until now, prevented a thorough study of their species status using modern systematic tools. Prior to this study, nothing was known about their biogeographic history.

*Dipodomys phillipsii* belongs to the rodent family Heteromyidae (kangaroo rats, pocket mice, and their allies), and *Peromyscus difficilis* is another cricetid rodent. Both are Mexican endemics with similar distributions. Populations of both species occur in the arid and semi-arid plains and low hills in the Oriental Basin and the Mexican Plateau, however *P. difficilis* has disjunct populations at higher elevations in the pine forests of the TMVB. Both species are divided into several subspecies that can be distinguished primarily by where they occur, plus a few qualitative morphological characters, such as body size and fur coloration. Until this study, neither species has been studied using modern systematic techniques and analyses.

My survey of mammals of the Oriental Basin and Mexican Plateau was carried out between 2006 and 2010 and, thanks to a collecting permit granted by the Mexican government to F. Cervantes on my behalf, I was able to obtain multiple samples, including fresh tissues, for each of the four study taxa. Using the tissues I collected plus other tissues generously donated to me by colleagues in multiple museums in Mexico and the U.S., I had the opportunity to examine, for the first time, phylogenetic and
phylogeographic relationships among the different populations and subspecies of the four study taxa using mitochondrial and nuclear markers analyzed using maximum likelihood and Bayesian approaches. Use of molecular markers under a coalescent framework also allowed me to estimate divergence dates within each taxon, which placed the evolutionary history of these Mexican endemics into a larger, historical-biogeographical context and provided the opportunity to test the role of geographic barriers and climate shifts in generating the pattern of evolutionary relationships we see today in these species.

1.1 Literature Cited


CARTRON, J. L., G. CEBALLOS, AND R. S. FELGER. 2005. Biodiversity, ecosystems, and


CHAPTER 2
MOLECULAR PHYLOGENETICS AND BIOGEOGRAPHY OF A RARE ENDEMIC: NELSON’S WOODRAT (NEOTOMA LEUCODON NELSONI) IN THE ORIENTAL BASIN OF MEXICO

2.1 INTRODUCTION

*Neotoma nelsoni* (Nelson’s woodrat) is a rare, monotypic, and endemic Mexican mammal, with only a handful of specimens housed in museum collections worldwide (González-Ruíz et al. 2006, Hall 1981). This species has been collected at only four localities in the states of Puebla and Veracruz (Acosta and Fernández 2009; Falcón-Ordaz et al. 2010; Goldman 1905; González-Christen et al. 2002; González-Ruíz et al. 2006), and >200 kilometers separate *N. nelsoni* from the nearest population of its putative sister species, *N. leucodon*, in the state of Hidalgo, Mexico (Fig. 2.1; González-Ruíz et al. 2006).

*Neotoma nelsoni* was originally described by E. A. Goldman in 1905 based on 11 specimens (the holotype and 10 paratypes) collected in the vicinity of Perote, Veracruz. Goldman differentiated *N. nelsoni* from *N. leucodon* by presence of a palatine bone with a short posterior spine, nasals that are more wedge-shaped and pointed posteriorly, and a tail that is indistinctly bicolored and nearly unicolored near the tip (Goldman 1905; González-Ruíz et al. 2006). The morphological differentiation and geographic isolation of *N. nelsoni* led Goldman (1905) to recognize it as a distinct species, presumably related to the much more widespread *N. leucodon*.

Hall and Genoways (1970) used morphological evidence to place *N. nelsoni* in the *N. albigula* species group, which included *N. albigula* (with 14 subspecies including the current *N. leucodon*), *N. palatina*, *N. varia*, and *N. nelsoni*. *Neotoma palatina*, a Mexican
Fig. 2.1.—Geographical distribution of the *Neotoma micropus* species group. Asterisks show the approximate collection locality of the two samples of *N. leucodon*. Localities are listed in the methods section (redrawn from Edwards et al. 2001; González-Ruíz et al. 2006; and Hall 1981).
endemic restricted to the canyon of the Río Bolaños, its tributaries, and immediately adjacent uplands in Jalisco (Hall and Genoways 1970), is still recognized as a valid species (Musser and Carleton 2005). *Neotoma varia*, also a Mexican endemic restricted to Isla Dátil off the coast of Sonora, was considered inseparable from *N. albigula* by Bogan (1997), who reduced it to subspecies status within *N. albigula*.

Several studies have surveyed genetic relationships in *Neotoma*, yet none of these, to date, has included a specimen of *N. nelsoni*. Planz et al. (1996) examined mitochondrial DNA restriction site polymorphism in *Neotoma* and suggested that *N. a. leucodon* may represent a cryptic species within *N. albigula*. Edwards et al. (2001) examined variation in the mitochondrial cytochrome *b* gene and, in agreement with Planz et al. (1996), concluded that *N. albigula* consists of two species, *N. albigula* in the southwestern United States and northwestern Mexico, and *N. leucodon* in the southcentral United States and northcentral Mexico (Fig. 2.1). Edwards et al. (2001) further determined that *N. albigula* and *N. goldmani* (endemic to the Mexican altiplano) are allied with the *N. floridana* species group (which also includes *N. floridana* and *N. magister*), whereas *N. leucodon* is allied with the *N. micropus* species group (which includes *N. micropus* and, possibly, *N. palatina*). Building on the study by Edwards et al. (2001), Edwards and Bradley (2002) examined cytochrome *b* variation in taxonomically and geographically larger samples of *Neotoma* and recovered the same species groupings. Most recently, a study of variation in four mitochondrial and four nuclear genes by Matocq et al. (2007) confirmed the *Neotoma* species groups defined by Edwards et al. (2001). Because specimens of *N. nelsoni* and *N. palatina* were not included in any of
these molecular studies, the taxonomic status and phylogenetic position of these species remain unclear.

During a 2007 survey of mammals of the Oriental Basin of Mexico, I trapped a specimen of *N. nelsoni* in the municipality of Perote, Veracruz. This capture affords the opportunity to examine, for the first time, phylogenetic relationships between *N. nelsoni* and its congener based on molecular markers. Use of molecular markers also facilitates calculation of estimated divergence dates within the clade containing *N. nelsoni*, which places the evolutionary history of this rare Mexican endemic into a larger, historical biogeographical context.

2.2 MATERIALS AND METHODS

**Sampling.**—The female specimen of *Neotoma nelsoni* (LSUMZ 36663) was trapped on June 22, 2007, in Veracruz (3 km S El Frijol Colorado, Municipality of Perote, 19.5723, -97.3835, 2,437 m; Fig. 2.2) under the authority of Mexican collecting permit FAUT-0002 issued to F. A. Cervantes. Traps were set in a dry plain with almost no vegetation and in low, rocky hills dominated by yucca (*Yucca* sp.), agave (*Agave* sp.), prickly pear (*Opuntia* sp.), and other cacti. The specimen of *N. nelsoni* was captured in the latter habitat. All mammal specimens were handled in accordance with guidelines approved by the American Society of Mammalogists (Kelt et al. 2010, Sikes et al. 2011).

DNA sequences generated by Edwards et al. (2001) and Matocq et al. (2007) for 14 species of *Neotoma* were downloaded from GenBank. The samples include one individual per species (unless indicated otherwise), and GenBank numbers are listed in the following order: *Cytb*; *12S*; *16S*. Taxa represented were *N. albigula* (DQ179707; DQ179757; DQ179857), *N. cinerea* (DQ179705; DQ179755; DQ179855), *N. floridana*
Fig. 2.2.—Map of central Mexico (inset modified from http://gaia.inegi.org.mx/mdm5/viewer.html) showing the known geographic distribution of Nelson’s woodrat, *Neotoma leucodon nelsoni* (shaded area in main map). The shaded area in the inset shows the extent of the Oriental Basin. The specimen examined in this study was collected at locality 1 in the inset (Falcón-Ordáz et al. (2010; this study). The three other known collection localities of *N. leucodon nelsoni* are based on reports by Goldman (1905; locality 2), González-Christen et al. (2002; locality 3), González-Ruíz et al. (2006; locality 4).
(DQ179669; DQ179719; DQ179819), *N. fuscipes* (DQ179672; DQ179722; DQ179822), *N. goldmani* (DQ179677; DQ179727; DQ179827), *N. isthmica* (DQ179678; DQ179728; DQ179828), *N. lepida* (DQ179681; DQ179731; DQ179831), *N. leucodon* (*n* = 2; DQ179665; DQ179715; DQ179815 from Texas, and DQ179689; DQ179739; DQ179839 from Durango), *N. macrotis* (DQ179691; DQ179741; DQ179841), *N. magister* (DQ179706; DQ179756; DQ179856), *N. mexicana* (DQ179695; DQ179745; DQ179845), *N. micropus* (DQ179668; DQ179718; DQ179818 from Texas, and DQ179698; DQ179748; DQ179848 from New Mexico), *N. picta* (DQ179701; DQ179751; DQ179851), and *N. stephensi* (DQ179702; DQ179752; DQ179852).

Sequences generated by Edwards et al. (2001) and Matocq et al. (2007) for *Hodomys alleni* (DQ179660; DQ179710; DQ179810), *Peromyscus attwateri* (DQ179661; DQ179711; DQ179811), *Ototylomys phyllotis* (DQ179664; DQ179714; DQ179814), *Tylomys nudicaudus* (DQ179662; DQ179712; DQ179812), and *Xenomys nelsoni* (DQ179663; DQ179713; DQ179813) were included as outgroups. Collection localities for all of the above specimens are available in Edwards et al. (2001) and Matocq et al. (2007).

Laboratory protocols.—Total genomic DNA was extracted from fresh tissue using a commercial kit (DNeasy Blood and Tissue Kit; Qiagen Inc., Valencia, California), and 3 mitochondrial genes were sequenced: cytochrome-*b* (*Cytb*), 12S ribosomal RNA (*12S*), and 16S ribosomal RNA (*16S*). Sequences were amplified by PCR (Saiki et al. 1988) using the following universal primers developed for rodents: MVZ-05 and H15915 for *Cytb* (Irwin et al. 1991); 12S L82 and 12S H900 for *12S* (Nedbal et al. 1994); and 16Sa and 16Sb for *16S* (Matocq et al. 2007). The following PCR parameters were used to
amplify genes: initial denaturation at 95°C for 2 min, followed by 27 cycles of
denaturation at 95°C for 1 min, annealing at 49°C for 1 min, and extension at 72°C for 2
min, with a 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 (2%) gels were
used to visualize amplified products. PCR products were purified using ExoSAP-IT
(Affymetrix, Santa Clara, California). DNA sequencing was performed for both light and
heavy strands with a Big Dye Terminator v1.1, v3.1 in an automated 3100 Genetic
Analyzer (Applied Biosystems, Foster City, California) at the Museum of Natural
Science, Louisiana State University. Sequences generated in this analysis (Cytb, 12S, and
16S.) were submitted to GenBank.

Data analysis.—Editing and alignment of sequences and matrix manipulations
were performed in Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Michigan).
Sequences were verified manually, and authenticity of the gene was confirmed by amino
acid translation and BLAST searches in GenBank

To enable comparison of our results with those of previous studies, raw sequence
divergence values for the Cytb gene were corrected using the Kimura 2-parameter
substitution model (Kimura 1980) in PAUP* (Phylogenetic Analysis using Parsimony,
version 4.0b, Swofford 2003).

Phylogenetic analyses were carried out under a maximum likelihood (ML)
framework in PAUP* and PhyML 3.0 (Guindon and Gascuel 2003). Analyses based on
Bayesian inference (BI) were conducted using MrBayes (version 3.2-cvs; Huelsenbeck
and Ronquist 2001; Ronquist and Huelsenbeck 2003). In all analyses, variable nucleotide
positions were considered unordered, discrete characters with 4 possible states (A, C, G, and T). Best-fit models for ML and BI analyses were evaluated using the Akaike Information Criterion and the program jModeltest 0.1.1 (Akaike 1973; Guindon and Gascuel 2003; Posada 2008). The HKY+I+G model was selected for the Cytb data, and the TIM2+I+G model was selected for the 12S and 16S genes. ML clade support was assessed with 500 bootstrap replicates (Felsenstein 1985) in PAUP*, and clade support in BI analyses was evaluated using posterior probabilities (pp).

Tree searches in the ML analyses were performed with the starting trees obtained via 100 random, stepwise additions followed by tree-bisection-reconnection (TBR) 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 $10^6$ generations in which trees were sampled every $10^3$ generations, resulting in $10^4$ samples for each run. A majority-rule consensus tree was constructed using the final $2 	imes 10^4$ trees. The analysis was stopped when the average standard deviation of split frequencies approached zero, and convergence also was assessed using Tracer 1.5 (Rambaut and Drummond 2007). The combined data set was analyzed in a partitioned way (genes and model parameters) to allow for independent convergence on optimal values for each component (Ronquist and Huelsenbeck 2003). Results from each gene were analyzed separately to evaluate potential conflict among gene trees. Nodes were considered well supported if there was >80% bootstrap support in ML analyses or >95% pp in BI analyses.

Analysis of divergence times.—The program *BEAST version 1.6.0 (Drummond and Rambaut 2007) was used to estimate the time of divergence between N. nelsoni and
other members of the *N. micropus* species group. A Yule tree prior was used, implicitly considering the gene tree to represent the species tree, and the HKY+I+G model was selected as the substitution model. To improve search efficiency, monophyly of the genus *Neotoma* was enforced. A relaxed clock with a lognormal distribution allowing rate variation among sites was used. Chains were run for $2^7$ generations, sampling the parameter every $10^3$ generations. Convergence statistics were checked for effective sample sizes using Tracer version 1.5 (Rambaut and Drummond 2007). Consensus trees were generated from the resulting $2 \times 10^4$ trees using TreeAnnotator version 1.6.0 (Rambaut and Drummond 2009) after elimination of the first 25% as burn-in.

Three fossil-based dates were used to calibrate the *BEAST* analysis. The 1st documents separation of the *Neotoma* lineage from ancestral cricetine stock in the upper (late) Miocene, during a period that lasted from approximately 11.6 to 5.3 MYA (Hibbard 1966). The 2nd and 3rd fossil-based dates establish minimum ages for *N. albigula* and *N. mexicana*, respectively, at the beginning of the Pleistocene (ca. 2.5 MYA; Álvarez 1969; Birney 1973, 1976; Dalquest and Stangl 1984; Harris 1984; Jakway 1958; Logan and Black 1979; Murray 1957; Schultz and Howard 1935; Van Devender et al. 1977). To account for uncertainty in the fossil-based calibrations, the dates were modeled as lognormal distributions rather than point calibrations (Ho and Phillips 2009).

2.3 Results

The final dataset consisted of 1,140 base pairs (bp) of the *Cytb* gene, 413 bp of the *12S* gene, and 561 bp of the *16S* gene for a total of 2,114 bp. *Cytb* divergence values (Table 2.1) show the *N. nelsoni* specimen from Veracruz, Mexico to be most similar genetically to the specimen of *N. leucodon* from Durango, Mexico (3.3% corrected
sequence divergence). Surprisingly, the two specimens of *N. leucodon*, one from Durango and the other from Texas, are only 5.5% genetically similar at the *Cyth* locus. *Neotoma nelsoni* exhibits >12% sequence divergence when compared to all other species of *Neotoma* in the dataset (*N. albigula, N. mexicana, and N. micropus*).

ML and BI analyses of the 12S gene indicated different topologies, but both trees were poorly resolved and had universally low branch support (trees not shown). Similar analyses of the 16S dataset recovered a moderately supported clade (75% bootstrap; 0.96 pp) that includes all members of the *N. micropus* species group (as defined by Matocq et al. 2007) plus the *N. nelsoni* sample. An internal clade with low branch support (50% bootstrap; <0.60 pp) grouped *N. nelsoni* with the two *N. leucodon* samples, but relationships among the 3 taxa were unresolved (trees not shown, but are available on request).

ML and BI analyses of the *Cyth* gene (Fig. 2.3) recovered topologies that were almost identical to those reported by Matocq et al. (2007). The *N. micropus* species group of Matocq et al. (2007) was recovered with strong nodal support (100% bootstrap; 1.00 pp), and *N. nelsoni* was included as a member of this group. Within the *N. micropus* species group, *N. nelsoni* clustered with the two samples of *N. leucodon* (100% bootstrap; 1.00 pp), and within this trio of taxa, *N. nelsoni* grouped with the specimen of *N. leucodon* from Durango (92% bootstrap; 0.99 pp) to the exclusion of the sample of *N. leucodon* from Texas (Fig. 2.3).

Although trees based on the individual genes showed no topological conflicts, a partitioned homogeneity test indicted that the genes could not be combined. As a result,
Table 2.1.—Cytochrome-\textit{b} sequence divergence values (Kimura 2-parameter model) between and within species of the \textit{Neotoma micropus} and \textit{N. floridana} species groups.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Cytb sequence divergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{N. nelsoni}–\textit{N. leucodon} (average)</td>
<td>4.17%</td>
</tr>
<tr>
<td>\textit{N. nelsoni}–\textit{N. leucodon} (Durango)</td>
<td>3.28%</td>
</tr>
<tr>
<td>\textit{N. nelsoni}–\textit{N. leucodon} (Texas)</td>
<td>5.07%</td>
</tr>
<tr>
<td>\textit{N. leucodon} (Durango)–\textit{N. leucodon} (Texas)</td>
<td>5.52%</td>
</tr>
<tr>
<td>\textit{N. nelsoni}–\textit{N. micropus}</td>
<td>12.83%</td>
</tr>
<tr>
<td>\textit{N. nelsoni}–\textit{N. albigula}</td>
<td>14.38%</td>
</tr>
<tr>
<td>\textit{N. nelsoni}–\textit{N. mexicana}</td>
<td>13.50%</td>
</tr>
</tbody>
</table>
Fig. 2.3.—Cladogram showing the placement of Nelson’s woodrat, *N. leucodon nelsoni*, within the genus *Neotoma* and the *N. micropus* species group (shaded box) based on a maximum likelihood (ML) analysis of cytochrome-\(b\) sequences. A Bayesian analysis of the same data yielded a tree with identical topology. Numbers above branches indicate ML bootstrap support, and numbers below branches indicate Bayesian posterior probabilities. The specimen labeled “*N. leucodon (D)*” is from Durango, and “*N. leucodon (T)*” is from Texas.
Fig. 2.4.—Cladogram showing placement of Nelson’s woodrat, *N. leucodon nelsoni*, within the genus *Neotoma* and the *N. micropus* species group (shaded box) based on a partitioned maximum likelihood (ML) analysis of 3 mitochondrial genes (*Cytb*, *12S*, and *16S*). A Bayesian analysis of the same data yielded a tree with identical topology. Numbers above branches indicate ML bootstrap support, and numbers below branches indicate Bayesian posterior probabilities.
the three sequence datasets were analyzed in a partitioned analysis. To save computational time, only members of the *N. micropus* and *N. floridana* species groups were included in these analyses. ML and BI analyses of the partitioned data (Fig. 2.4) showed topologies identical to the *Cytb* tree (Fig. 2.3) and confirmed monophyly of both species groups (100% bootstrap and 1.00 pp for both clades). The sister relationship between *N. nelsoni* and the *N. leucodon* sample from Durango also was confirmed (97% bootstrap; 1.00 pp; Fig. 2.4).

**Analysis of divergence times.**—Except for the position of *N. stephensi* (which is not relevant to the present study), the tree obtained in the *BEAST analysis* (Fig. 2.5) was identical to the BI tree published by Matocq et al. (2007; their Fig. 4). The Tracer analysis of *BEAST* output files confirmed a high effective sample size (>3,500) for all parameters. Although error estimates are large for most nodes, results suggest that the *N. micropus* and *N. floridana* species groups diverged near the end of the Miocene (ca. 6.7 mya) and most speciation events within these species groups took place in Pliocene or early Pleistocene times (ca. 4.6 to 2.0 mya; Fig. 2.5). The split between *N. leucodon* (Texas sample) and the *N. nelsoni + N. leucodon* (Durango) clade was placed at approximately 3 mya (late Pliocene), and divergence of *N. nelsoni* from *N. leucodon* (Durango) is estimated to have occurred approximately 2 mya during the early Pleistocene.

**2.4 DISCUSSION**

**Species status of *N. nelsoni*.**—The discovery that *N. nelsoni* is phylogenetically closer to *N. leucodon* from Durango than the latter specimen is to *N. leucodon* from Texas calls into question the monophyly of *N. leucodon* and the species status of *N.
Fig. 2.5.—Phylogeny and chronogram of selected species of the genus *Neotoma* (and outgroups), and estimated divergence times calculated from *BEAST* analysis. Numbers at nodes are mean divergence dates (mya) and gray bars show the 95% credibility intervals for nodes of interest. Black circles identify calibration points based on fossil evidence. The specimen labeled “*N. leucodon* (D)” is from Durango, and “*N. leucodon* (T)” is from Texas.
Continued recognition of *N. nelsoni* at the species level would require elevation of the northern clade of *N. leucodon* to species level (as *N. warreni* Merriam, 1908), but there is no compelling evidence to warrant this action. The *Cytb* sequence divergence measured between the Texas and Durango samples of *N. leucodon* in this study (5.5%; Table 2.1) is within the range of values reported for conspecific populations by Bradley and Baker (2001). The morphological characters used to distinguish *N. nelsoni* from *N. leucodon* (modest differences in pelage quality and coloration and minor, qualitative differences in a few cranial bones; González-Ruíz et al. 2006) are on the order of characters normally used to distinguish among subspecies in other rodents. Given the current absence of diagnostic morphological characters to distinguish *N. nelsoni* from *N. leucodon* and the relatively low level of *Cytb* sequence divergence between the 2 forms (3.3%; well within the range of values reported for conspecific populations by Bradley and Baker, 2001), I take a conservative taxonomic approach and recognize *N. nelsoni* as a subspecies of *N. leucodon* (as *N. leucodon nelsoni* Goldman 1905). Designation of subspecific epitaphs for all other populations of *N. leucodon* must await a large-scale assessment of geographic variation within the species.

Estimates of divergence times.—Early hypotheses about the diversification of the North American mammalian fauna suggested that Pleistocene glacial and interglacial cycles were major generators of species-level diversity (e.g., Findley 1969; Orr 1960). Consistent with this widely held contention, Zimmerman and Nejtek (1977) used data obtained by protein electrophoresis to estimate that basal divergence events leading to the current species *N. albigula*, *N. floridana*, and *N. micropus* occurred between 112,000 and 155,000 years ago. Zimmerman and Nejtek (1977) implicated vegetational changes that
occurred in response to Pleistocene glaciations as the causal force driving speciation in this clade.

Recently, new distributional, paleontological, and molecular evidence has modified the “Pleistocene paradigm,” assigning a prominent role to geologic events that took place during Miocene and Pliocene times (Hafner and Riddle 1997; Riddle 1995; Riddle et al. 2000; Vrba, 1992). For example, estimates of divergence times calculated in this study suggest that the initial split between the *N. micropus* and *N. floridana* species groups occurred near the end of the Miocene (approximately 6.7 mya; Fig. 2.5), long before the major climatic fluctuations of the Pleistocene. Mean estimates of divergence events within the *N. micropus* and *N. albigula* species groups also are pre-Pleistocene, with diversification in both groups beginning in early Pliocene (ca. 4.5 mya). The divergence between *N. leucodon nelsoni* and the *N. leucodon* sample from Durango is the only event postulated to have taken place during the Pleistocene (Fig. 2.5).

If, as these data suggest, major divergence events in the *N. micropus* and *N. floridana* species groups took place during Miocene and Pliocene times, then geological and climatic events of those times probably played major roles, either directly or indirectly, in this diversification. The Trans-Mexico Volcanic Belt (TMVB) began to form in the Miocene (Ferrusquía-Villafranca 2007) and is still active today. Presently, it consists of a large, but somewhat fragmented, chain of mountains extending from the Pacific coast to the Gulf of Mexico at the latitude of Mexico City. Fragmentation of the TMVB through time created a large number of interior lakes, some of which persist today, whereas others have dried up, giving rise to small pockets of alkaline desert inside the TMVB. The Oriental Basin, home to at least one population of *N. leucodon nelsoni*
and several endemic animals and plants, is one such alkaline desert near the eastern end of the TMVB (Ferrusquía-Villafranca 2007; Morán-Zenteno 2004; Shreve 1942; Viniegra 1992).

The TMVB is widely recognized as a generator of biological diversity in Mexico, and the uplift of the TMVB and associated creation of interior lakes and small patches of desert may have isolated ancestral populations of many organisms, including the common ancestor of the *N. leucodon* populations now in Durango and *N. leucodon nelsoni* in the Oriental Basin region well before the Pleistocene. Once isolated, these populations would be subject to the many geologic and climatic perturbations of the late Miocene, Pliocene, and early Pleistocene, resulting in a mixed pattern of diversification, in which some lineages diverge well before the Pleistocene, whereas others respond to the well-known geologic, glacial, and climate changes of the Pleistocene. It seems that the evolutionary history of *N. leucodon nelsoni* may be yet another example of the isolating force of the TMVB (Demastes et al. 2002; Douglas et al. 2010; Hewitt 2001; Mateos et al. 2002; McCormack et al. 2008; Mulcahy and Mendelson 2000; Zink and Blackwell 1998).

**Natural history and conservation status of *N. leucodon nelsoni*.**—Given the rarity and isolation of *N. leucodon nelsoni* populations, a few comments on the natural history and conservation status of this taxon are in order. Only 14 specimens of Nelson’s woodrat are known to date. Goldman (1905) described the species based on 11 specimens collected in 1893, and more than a century passed before 3 additional specimens were collected, including the specimen used in this study (González-Christen et al. 2002; González-Ruíz et al. 2006). The specimens examined by Goldman (1905)
were collected near Perote, Veracruz, but it is difficult to determine the habitat at their site of capture because Goldman used the locality “Perote, Veracruz” for all specimens collected in the general vicinity of Perote, which contains a wide variety of habitat types (González-Ruíz et al. 2006). González-Christen et al. (2002) collected a single specimen of *N. nelsoni* in a tropical rain forest about 30 km SE of the type locality, and González-Ruíz et al. (2006) reported another specimen collected in a mountainous area with cloud forests and coffee plantations approximately 40 km S of the type locality. The specimen used in this study was collected in dry, low hills of volcanic origin surrounded by xeric shrubs and cacti approximately 15 km W of the type locality. Given the wide variety of habitats in which the few known specimens of *N. leucodon nelsoni* have been captured, it appears that this subspecies has high environmental plasticity (as suggested by González-Ruíz et al. 2006) and can live in a broad array of vegetational associations.

Despite its high environmental plasticity, *N. leucodon nelsoni* seems to have a clear association with the Oriental Basin (“Cuenca Oriental”; Fig. 2.2), and all known specimens of *N. leucodon nelsoni* have been collected in or around this xeric basin. The Oriental Basin is a semiarid, endorheic (closed drainage) basin that extends over portions of the Mexican states of Puebla, Tlaxcala, and Veracruz. This relatively small (ca. 5,000 km²) basin is characterized by alkaline grasslands, bunch grasses, and aridland scrub in the valleys, and coniferous forests in the surrounding mountains (Valdés and Ceballos 1997). The basin and surrounding areas support several endemic taxa of plants and animals, including at least 4 mammal species exclusive to the Oriental Basin region: the Oriental Basin pocket gopher (*Cratogeomys fulvescens*; Hafner et al. 2005), the Perote ground squirrel (*Xerospermophilus perotensis*; Best and Ceballos 1995), the Perote
deermouse (*Peromyscus bullatus*; González-Ruiz and Álvarez-Castaneda 2005), and Nelson’s woodrat (*N. leucodon nelsoni*; González-Ruiz et al. 2006; this study).

The specimen of *N. leucodon nelsoni* used in this study was collected in sympatry with several endemic and non-endemic rodent species, including the Perote ground squirrel, Phillip’s kangaroo rat (*Dipodomys phillipsii*), silky pocket mouse (*Perognathus flavus*), western harvest mouse (*Reithrodontomys megalotis*), and rock mouse (*Peromyscus difficilis*). Two species of rattlesnakes (*Crotalus molossus nigrescens* and *C. scutulatus salvini*) were collected near the capture locality, and a third species (*C. ravus ravus*) known from this region (Camarillo 1998) also may prey on woodrats and other rodents in the community.

A new genus of nematode, *Lamotheoxyuris*, was described from the specimen of *N. leucodon nelsoni* used in this study (Falcón-Ordaz et al. 2010), and three species of fleas were collected from this specimen, one of them (*Aniomiopsyllus perotensis*) new to science (Acosta and Fernández 2009).

Despite intensive collecting efforts by several research parties working in and around the Oriental Basin (Hall and Dalquest 1963; González-Christen et al. 2002; González-Ruiz et al. 2006; this study), only a few specimens of *N. leucodon nelsoni* are known to science. *Neotoma leucodon nelsoni* is listed (as *N. nelsoni*) as critically endangered by the International Union for Conservation of Nature (IUCN 2011) but, ironically, the scarcity of information about the density and distribution of *N. leucodon nelsoni* populations has caused this taxon to be excluded from the Mexican list of endangered species (Luiselli 2002). There is little doubt that *N. leucodon nelsoni* populations are threatened by continued and extensive conversion of natural habitats to
agriculture in the Oriental Basin region (González-Ruiz et al. 2006). New collecting
efforts are needed in the remaining areas of natural habitat to learn more about the
biology of *N. leucodon nelsoni* so as to protect it in the future.

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40


CHAPTER 3
PHYLOGENETICS AND BIOGEOGRAPHY OF THE MICROENDEMIC RODENT
XEROSPERMOPHILUS PEROTENSIS (PEROTE GROUND SQUIRREL) IN THE
ORIENTAL BASIN OF MEXICO

3.1 INTRODUCTION

The concept of endemism is central to the fields of biogeography and biological conservation, and areas with high numbers of endemic species, or “biodiversity hotspots,” 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 two 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 2002; Platnick 1991).

Mexico is widely known as a biologically megadiverse country and a biodiversity hotspot (Lamoreaux 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 Leon et al. 2007). In central Mexico, the highlands and arid valleys of the Trans-Mexico Volcanic Belt (TMVB) 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 TMVB lies the Oriental Basin (Cuenca Oriental; Fig. 3.1). By any definition of “area of endemism,” this semiarid, endorheic (closed drainage) basin, which covers portions of Puebla, Tlaxcala, and Veracruz, is an important area of endemism in North America. This relatively small (ca.
Fig. 3.1.—Geographical distribution of pygmy ground squirrels of the genus *Xerospermophilus* (modified from Hall 1981). Numbered dots show collection localities listed in Appendix 3.1 for *Xerospermophilus* specimens used in this study. Locality 8 and all localities outside shaded areas are collection sites for outgroup specimens (Appendix 3.1). The shaded area near locality 11 shows the approximate extent of the Oriental Basin (*Cuenca Oriental*).
5,000 km²) basin characterized by alkaline grasslands, bunch grasses, and aridland scrub in the valleys and coniferous forests in the surrounding mountains (Valdéz and Ceballos 1997) supports several endemic taxa of plants and animals, including at least four taxa of endemic mammals: the Oriental Basin pocket gopher (*Cratogeomys fulvescens*), the Perote deermouse (*Peromyscus bullatus*), Nelson’s woodrat (*N. nelsoni*), and the Perote ground squirrel (*Xerospermophilus perotensis*; Best and Ceballos 1995; González-Ruíz and Álvarez-Castaneda 2005; González-Ruíz et al. 2006; Hafner et al. 2005).

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. 3.1; Howell 1938).

In his classic 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 eight subgenera: *Ammospermophilus*, *Callospermophilus*, *Citellus*, *Ictidomys*, *Notocitellus*, *Otospermophilus*, *Poliocitellus*, and *Xerospermophilus*. Early morphological and chromosomal studies suggested a close relationship between *Spermophilus perotensis* and *S. spilosoma* (Howell 1938; Uribe and Ahumada 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* (Harrison et al. 2003; Herron et al. 2003).
The taxonomic status and systematic affinities of \textit{S. perotensis} were not investigated again until molecular studies by Harrison et al. (2003) and Herron et al. (2004) confirmed the close affinity of \textit{S. perotensis} with \textit{S. spilosoma}. In fact, both molecular studies (using the same specimens of \textit{S. spilosoma} and based on the cytochrome-\textit{b} [\textit{Cytb}] gene) showed \textit{S. spilosoma} to be paraphyletic with respect to \textit{S. perotensis}, with \textit{S. s. pallescens} from Mexico sister to \textit{S. perotensis}, and \textit{S. s. marginatus} from Kansas sister to the \textit{S. s. pallescens + S. perotensis} clade. These same studies showed the \textit{S. perotensis + S. spilosoma} clade (subgenus \textit{Ictidomys}) to be sister to the \textit{S. mohavensis + S. tereticaudus} clade (subgenus \textit{Xerospermophilus}), with prairie dogs (\textit{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 \textit{Xerospermophilus} to full generic status. In \textit{Xerospermophilus}, Helgen et al. (2009) included the 4 species of pygmy ground squirrels adapted to arid and semi-arid conditions, \textit{X. mohavensis}, \textit{X. tereticaudus}, \textit{X. spilosoma}, and \textit{X. perotensis}. In view of the potential paraphyly of \textit{X. spilosoma} (Harrison et al. 2003; Herron et al. 2003), Helgen et al. (2009) recommended future research into species-level boundaries in the \textit{spilosoma-perotensis} complex.

Despite previous morphological and molecular studies of the systematic status of \textit{X. perotensis} and allied taxa, several uncertainties remain with respect to the species status of \textit{X. perotensis}, monophyly of \textit{X. spilosoma}, and the timing of diversification events within the genus \textit{Xerospermophilus} relative to major geological and climatic events. Each of these issues is explored in this analysis using newly acquired samples of
3.2 Material and Methods

**Sampling.**—Tissue samples of *Ictidomys mexicanus* (*n* = 1 individual), *I. parvidens* (*n* = 2), *I. tridecemlineatus* (*n* = 4), *Xerospermophilus 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 3.1). In addition to the DNA sequences generated in this study, 32 sequences were downloaded from Genbank for use in the molecular analyses (Appendix 3.1). 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 *Sciurus niger* (in the growth hormone receptor [GHR] analysis). The collection and processing of samples was 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 two nuclear genes (*GHR* and *IRBP*), and two mitochondrial genes (*Cytb* and 12S) were sequenced for subsequent analysis. The genes were amplified by PCR (Saiki et al. 1988) using the following universal primers developed for rodents: GHR1f and GHRend1f for *GHR* (Jansa et al. 2009); IRBP-A and IRBP-B for *IRBP* (Stanhope et al. 1992); MVZ-05 and H15915 for *Cytb* (Irwin et al. 1991); and 12S L82 and 12S H900 for 12S (Nedbal et al. 1994). PCR amplification of the *GHR* gene was performed under the
following parameters: initial denaturation at 94°C for 5 min followed by 34 cycles of
denaturation at 94°C for 15 sec, 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 sec, annealing at 58°C for 20 sec, extension of 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. PCR
products were purified using either Polyethylene Glycol (PEG) or ExoSAP-IT
(Affymetrix, Santa Clara, California). DNA sequencing was performed for both light and
heavy strands with a Big Dye Terminator v1.1, v3.1 in an automated 3100 Genetic
Analyzer (Applied Biosystems, Foster City, California) at the Museum of Natural
Science, Louisiana State University. Editing and alignment of sequences and matrix
manipulations were performed in Sequencher 4.7 (Gene Codes Corporation, Ann Arbor,
Michigan), MacClade (Maddison and Maddison 2000), and Mesquite (Maddison and
Maddison 2010). Sequences were verified manually, and authenticity of the gene was
confirmed by amino acid translation and BLAST searches in Genbank

Estimates of genetic divergence.—To enable comparison of my results with those
of previous studies, sequence divergence values for the Cytb gene were corrected using
the Kimura 2-parameter substitution model (Kimura 1980) in PAUP* 4.0b10
(Phylogenetic Analysis using Parsimony, version 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 affects 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 maximum likelihood (ML) in PAUP*. Analysis of the *Cytb* data included 41 specimens of 14 species for which complete *Cytb* sequences were available. Phylogenetic analysis of the *12S* gene included 17 specimens of 8 species, analysis of *GHR* included 14 specimens of 6 species, and analysis of *IRBP* included 18 specimens representing 8 species (Appendix 3.1). 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). Variable nucleotide positions were considered unordered, discrete characters with 4 possible states (A, C, G, T). Best-fit models for ML and BI analyses were evaluated using the Akaike Information Criterion (AIC) and the program jModeltest 0.1.1 (Guindon and Gascuel 2003; Posada 2008). The following models were selected for *Cytb*, *12S*, *GHR*, and *IRBP* genes, respectively: TrN+G, TIM3+G, TPM3UF+I+G, and TPM3UF+I. ML clade support was assessed with 100 bootstrap (bs) replicates in PAUP* and PhyML 3.0, and clade support in the BI analyses was evaluated using posterior probablilities (pp).
ML analyses were performed with the starting trees obtained from 100 random, stepwise additions followed by tree-bisection-reconnection (TBR) 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 two independent runs of $10 \times 10^6$ 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 $18 \times 10^3$ trees. The analysis was stopped when the average standard deviation of split frequencies approached zero and convergence was reached, as determined using Tracer version 1.5 (Rambaut and Drummond 2007). 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, *Cytb* versus *12S*, *Cytb* versus *IRBP*, and *12S* versus *IRBP* genes. Nodes were considered well supported if there was $>80\%$ bootstrap support in ML analyses or $>95\%$ posterior probability in BI analyses.

**Estimates of divergence times.**—The program *BEAST* version 1.6.0 (Bayesian evolutionary analysis sampling trees; Drummond and Rambaut 2007) was used to generate estimates of the timing of divergence of *X. perotensis* from related species. *BEAST* analyses were carried out using a Yule tree prior and implicitly considering the *Cytb* gene tree to represent the species tree. The estimate was calibrated using a dated fossil (Goodwin 1995; Harrison et al. 2003; Pizzimenti 1975) to constrain the minimum date for separation of *Cynomys* from *Xerospermophilus* to 2.7 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. Chains were run for $2 \times 10^7$ generations, sampling the parameter every $10^3$ generations. Convergence statistics were checked for effective sample sizes using Tracer version 1.5. 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.

3.3 Results

Analyses of DNA sequences involved a total of 3,403 base pairs (bp), including 1,141 bp of \textit{Cytb}, 736 bp of \textit{12S}, 910 of \textit{GHR}, and 616 bp of \textit{IRBP}. ML and BI analyses using only \textit{Cytb} sequences from the 41 individuals with complete \textit{Cytb} sequences recovered trees with identical branch structure (Fig. 3.2). In these trees, there is strong support (pp = 1.00; bs = 100%) for monophyly of the genus \textit{Xerospermophilus}. Within \textit{Xerospermophilus}, \textit{X. perotensis} is shown to be sister to the \textit{X. spilosoma} sample from the state of San Luis Potosí on the Mexican Plateau (locality 12 in Fig. 3.1) to the exclusion of other samples of \textit{X. spilosoma} from Durango (locality 15), Kansas (14), and New Mexico (13). \textit{X. mohavensis} and \textit{X. tereticaudus} also are depicted as sister taxa. Among the many outgroups used in the analysis (listed above and in Appendix 3.1), the genus \textit{Cynomys} was found to be sister to \textit{Xerospermophilus}, although this relationship was not well supported (pp = 0.84 and bs = 79%) and therefore is not shown in Fig. 3.2.

\textit{Cytb} divergence values (Table 3.1) show the taxa included in Fig. 3.2 to be well differentiated genetically. \textit{X. perotensis} is 3.6% genetically divergent from the \textit{X. spilosoma} sample from San Luis Potosí, and these two populations together show an average \textit{Cytb} divergence of 6.4% from the other samples of \textit{X. spilosoma} from Durango,
Fig. 3.2.—Relationships among major clades of *Xerospermophilus* based on maximum likelihood and Bayesian analyses of Cytochrome-*b* sequences. Locality numbers (mapped in Fig. 3.1 and listed in Appendix 3.1) are indicated before taxon names. Numbers at nodes are estimated mean divergence dates (mya) calculated from *BEAST* analysis. Bars show 95% credibility intervals.
<table>
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<tr>
<th></th>
<th>X. perotensis</th>
<th>X. spilosoma San Luis Potosí</th>
<th>X. spilosoma Durango</th>
<th>X. spilosoma New Mexico, Kansas</th>
<th>X. mohavensis</th>
<th>X. tereticaudus</th>
<th>Cynomys</th>
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<tr>
<td>X. perotensis</td>
<td>–</td>
<td>3.6</td>
<td>5.2</td>
<td>7.9</td>
<td>10.0</td>
<td>11.5</td>
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<td>X. spilosoma</td>
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<td>6.0</td>
<td>6.4</td>
<td>10.6</td>
<td>12.0</td>
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<td>San Luis Potosí</td>
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<td>X. spilosoma</td>
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<td>X. spilosoma</td>
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<td>New Mexico, Kansas</td>
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<tr>
<td>X. mohavensis</td>
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<td>4.6</td>
<td>11.0</td>
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<td>X. tereticaudus</td>
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<td>14.2</td>
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<td>Cynomys</td>
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New Mexico, and Kansas. The \textit{X. perotensis} + \textit{X. spilosoma} clade (including all samples of \textit{spilosoma}) shows an average of 11.2\% sequence divergence from the \textit{X. mohavensis} + \textit{X. tereticaudus} clade and 11.8\% divergence from specimens of the genus \textit{Cynomys}. Independent BI and ML analyses of \textit{12S}, \textit{GHR}, and \textit{IRBP} sequences confirmed the \textit{Cytb} topology shown in Fig. 3.2, although nodal support values varied widely depending on the gene analyzed (trees not shown but available on request). In all analyses, the genus \textit{Xerospermophilus} was monophyletic and sister to \textit{Cynomys}, the sister species status of \textit{X. mohavensis} + \textit{X. tereticaudus} was confirmed with strong support, and the sister relationship between \textit{X. perotensis} and the \textit{X. spilosoma} sample from San Luis Potosí was recovered, but with low nodal support in the analyses of the nuclear genes (\textit{GHR} and \textit{IRBP}).

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 \textit{Xerospermophilus} (Fig. 3.3). In all partitioned analyses, the genus \textit{Xerospermophilus} was monophyletic with high support values and the sister status of \textit{X. perotensis} and \textit{X. spilosoma} (San Luis Potosí) was confirmed with high nodal support (pp $> 0.97$ and bs $= 100\%$).

Mean estimates of divergence times (Fig. 3.2) ranged from a low of 0.7 mya between \textit{X. perotensis} and \textit{X. spilosoma} (San Luis Potosí) to a high of 3.5 mya between the genera \textit{Cynomys} and \textit{Xerospermophilus}. All estimates of divergence times within the genus \textit{Xerospermophilus} place these events in the Pleistocene, with the possible exception of the split between the \textit{X. mohavensis} + \textit{X. tereticaudus} clade and the \textit{X. perotensis} + \textit{X. spilosoma} clade, which was estimated at 2.7 mya with a confidence interval extending from 4.3 to 1.3 mya (Fig. 3.2).
Fig. 3.3.—Relationships among major clades of *Xerospermophilus* based on a maximum likelihood analysis of *Cytb*, 12S, GHR, and IRBP sequences partitioned by gene. A Bayesian analysis of the same data yielded identical relationships. *Xerospermophilus spilosoma* from Durango (locality 15) was excluded from these analyses because only *Cytb* sequences were available for that specimen.
3.4 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 *X. spilosoma* sample 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 and Ahumada (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 3.2 and 3.3). 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 could also be argued that *X. perotensis* and *X. spilosoma* populations 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 3.1). 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. cabrerai* in this study (3.6%) lies within this range but is less than the “high genetic divergence” value of 5% suggested by Baker and Bradley (2006:654) to signal possible cryptic species. Morphologically, *X. perotensis* differs from *X. spilosoma* in ways that are usually used to distinguish among subspecies of rodents. For example, *X. perotensis* resembles *X. spilosoma 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. spilosoma 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 brain case, has auditory bullae that are broader and more flattened, and molariform teeth that are heavier than those of *X. s. spilosoma* (Best and Ceballos 1995; Hafner and Yates 1983; Helgen et al. 2009; Uribe 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 3.1) may signal presence of multiple cryptic species, but 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 semi-arid regions of central Mexico were relicts of a once continuous southern extension of
the Chihuahuan Desert. More recent research suggests that the arid and semi-arid lands of central Mexico were continuous until mid-late Miocene, when rise of the TMVB began to act as a barrier between populations of arid-adapted species (Ferrusquía et al. 2005; Ferrusquía and González 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 and Ahumada (1990) speculated that *X. spilosoma* stock was once widespread throughout the highlands of northern Mexico and were able to disperse southward because of continuous, dry habitats found in inter-montane 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 semi-arid habitats, including the Oriental Basin (Ferrusquía et al. 2005; Ferrusquía and González 2005; Hoffman and Jones 1970; Pizzimenti 1975).

The results of this study underscore the importance of three under-studied biogeographic regions of Mexico: the Oriental Basin (inhabited by *X. s. perotensis*), the Mexican Plateau (*X. s. cabrerai*), and the Bolsón de Mapimí (*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 semi-arid land in the TMVB (Caballero et al. 2003; Morán-Zenteno 1994). The Mexican Plateau formed as a result of uplifting of the Sierra Madre
Oriental, Sierra Madre Occidental, and TMVB, which created a dry, table land 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 (Fernandez et al. 2012; Neiswenter and Riddle 2010). Finally, the Bolsón de Mapimi, a closed desert basin located north of the Sierra de Zacatecas, 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*.

### 3.5 Literature Cited


59


CHAPTER 4

4.1 INTRODUCTION

Although most studies of desert mammals of North America have focused on species confined to the Chihuahuan, Peninsular, or Sonoran deserts, recent studies have begun to explore temporal aspects of species-level diversification in mammals whose distribution extends southward from the Mexican Plateau into the arid lands associated with the Trans-Mexico Volcanic Belt (TMVB; Fernández et al. in press; Neiswenter and Riddle 2010). This study focuses on one such species, the southern rock deermouse (*Peromyscus difficilis*), whose distribution in a wide variety of habitats (Fernández et al. 2010) affords an unusual opportunity to explore the effects of geology and climate on timing of phyletic diversification in a North American rodent species.

*Peromyscus difficilis* is endemic to Mexico and is found throughout the Mexican Plateau, Sierra Madre Oriental, and southward into north central Oaxaca (Fig. 4.1; Fernández et al. 2010; Hall 1981). Current taxonomy divides *P. difficilis* into five subspecies (Fernández et al. 2010; Hall 1981; Hoffmeister and de la Torre 1961; Fig. 4.1). Habitats occupied by *P. d. amplus*, *P. d. difficilis*, *P. d. petricola*, and *P. d. saxicola* include dry rocky areas, and oak-pine forests at higher elevations (2,000–2,400 m) and dry rocky hillsides with scattered juniper trees at lower elevations (1,500–1,900 m; Fernández et al. 2010). In contrast, *P. d. felipensis* is found in wet coniferous forests at high elevations (2,500–3,500 m) in mountains surrounding the Valley of
Fig. 4.1—Geographical distribution of *Peromyscus difficilis* and *P. nasutus* in Mexico and the United States (redrawn from Fernández et al. 2010 and Hall 1981). Numbered dots show collection localities listed in Appendix 4.1 for *P. nasutus* and the five currently recognized subspecies of *P. difficilis*. 
Mexico and in mountains near the city of Oaxaca (Goodwin 1954; Müdespacher-Ziehl et al. 2005; Navarro-Frías et al. 2007; Villa-Ramírez 1953).

Hoffmeister and de la Torre (1961) examined morphological variation in *P. difficilis* throughout its range and recognized eight subspecies, the current five plus three subspecies presently assigned to *P. nasutus* (*P. nasutus nasutus*, *P. n. griseus*, and *P. n. penicillatus*; Musser and Carleton 2005). Zimmerman et al. (1975, 1978) studied allelic differentiation at 23 protein loci in populations of *P. difficilis* from Zacatecas and Colorado and elevated *P. nasutus* to species status (supported by Avise et al. 1979; Carleton 1989). More recent studies of *Peromyscus* relationships based on the mitochondrial cytochrome-*b* gene (Durish et al. 2004; Bradley et al. 2007) have reaffirmed the species status of *P. difficilis* and *P. nasutus*.

Phylogeographic relationships among the subspecies of *P. difficilis* are not well understood. Whereas all populations examined to date have a chromosomal diploid number of 48, variation in fundamental number (FN) within the species (Arellano-Meneses et al. 2000; Müdespacher-Ziehl et al. 2005; Robbins and Baker 1981; Zimmerman et al. 1975) suggests possible presence of genetic variation that remains undiscovered.

In this study, phylogeographic relationships among the subspecies of *P. difficilis* and between *P. difficilis* and its close relatives are examined using nuclear and mitochondrial DNA sequences analyzed by maximum likelihood and Bayesian approaches. Estimates of divergence times based on Bayesian analyses of genetic data affords an unusual opportunity to test the role of geographic barriers and climate shifts in generating the pattern of evolutionary relationships we see today in this species.
4.2 MATERIALS AND METHODS

Sampling, amplification, and sequencing.—Two mitochondrial genes (cytochrome-b, Cytb, and 12S ribosomal RNA, 12S) and two nuclear genes (growth hormone receptor, GHR, and interphotoreceptor retinoid-binding protein, IRBP) were sequenced for this analysis. In the initial phase of the analysis, Cytb sequences were generated for P. d. amplus (n = 12 individuals), P. d. difficultis (n = 6), P. d. felipensis (n = 3), P. d. petricola (n = 4), P. d. saxicola (n = 3), and P. nasutus (n = 5). Following analysis of the Cytb data, representatives of each major clade were sequenced for 12S, GHR, and IRBP. Sequences for the 12S gene were generated for P. d. amplus (n = 4), P. d. difficultis (n = 4), P. d. felipensis (n = 1), P. d. petricola (n = 3), P. d. saxicola (n = 1), and P. nasutus (n = 3). GHR sequences were obtained for P. d. amplus (n = 6), P. d. difficultis (n = 1), P. d. felipensis (n = 1), P. d. petricola (n = 4), P. d. saxicola (n = 1), and P. nasutus (n = 2). Finally, IRBP sequences were generated for P. d. amplus (n = 6), P. d. difficultis (n = 2), P. d. felipensis (n = 1), P. d. petricola (n = 4), P. d. saxicola (n = 1), and P. nasutus (n = 2). Most of the tissue samples were collected in the field by the author following the guidelines for research on wild mammals approved by the American Society of Mammalogists (Kelt et al. 2010; Sikes et al. 2011). The remainder of the tissue samples were generously donated by museums (Appendix 4.1), and 22 additional sequences (including outgroup sequences for P. attwateri, P. nasutus, P. truei, and additional outgroups listed in Appendix 4.2) were downloaded from GenBank.

Extractions of genomic DNA were performed using a commercial kit (DNeasy Blood and Tissue Kit; Qiagen Inc., Valencia, California). Mitochondrial and nuclear genes were amplified by polymerase chain reaction using the following universal primers developed for rodents: MVZ-05 and H15915 for Cytb (Irwin et al. 1991); 12S L82 and 12S H900 for 12S (Nedbal et al. 1994); GHR1f and GHRend1f for GHR (Jansa et al. 2009); and IRBP-A and IRBP-B for IRBP
Thermal-cycling parameters for both mitochondrial genes were: initial denaturation at 95°C for 2 min, 27 cycles of denaturation at 95°C for 1 min, annealing at 49°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 7 min. Amplification parameters for GHR were: denaturation at 94°C for 5 min, 34 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 1 min, extension at 72°C for 1.5 min, and final extension for 10 min at 72°C. Parameters for IRBP were: denaturation at 95°C for 10 min, 27 cycles of denaturation at 95°C for 25 sec, annealing at 58°C for 20 sec, extension at 72°C for 1 min, and final extension for 10 min at 72°C. Agarose gels (2%) were used to visualize amplified products. Cleaning of the amplified products was performed with Polyethylene Glycol (PEG) and DNA sequencing was performed for both light and heavy strands using Big Dye Terminator v1.1, v3.1 (Applied Biosystems, Foster City, California) in an automated 3100 Genetic Analyzer (Applied Biosystems) at the Museum of Natural Science, Louisiana State University.

Data Analysis.—Editing and alignment of sequences were done with Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Michigan). The authenticity of each gene was confirmed by amino acid translation and by BLAST searches in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To enable comparison of our results with those of previous studies, average uncorrected sequence divergence values for the Cytb gene were corrected using the Kimura 2-parameter substitution model (Kimura 1980) in PAUP* (Phylogenetic Analysis using Parsimony, version 4.0b, Swofford 2003). This model has been used widely in studies of Cytb variation in mammals (Bradley and Baker 2001; Honeycutt et al. 1995).

Phylogenies were estimated using the maximum likelihood (ML) algorithms in PAUP* 4.0b (Swofford 2003) and PhyML 3.0 (Guindon and Gascuel 2003) and the Bayesian Inference
(BI) approach in MrBayes (version 3.2-cvs; Huelsenbeck and Ronquist 2001). Best-fit models for all analyses were selected using the Akaike Information Criterion (AIC; Akaike 1973) and the programs jModeltest 0.1.1 (Guindon and Gascuel 2003; Posada 2008), MrModeltest (version 2.2; Nylander 2004), and Modeltest (version 3.7; Posada and Buckley 2004; Posada and Crandall 1998). The TrN+G model was selected for Cytb, TIM2+G for 12S, TPM1uf+G for GHR, TPM3uf+I+G for IRBP, and TPM1uf+G for the partitioned analyses. Clade support was assessed with 500 bootstrap replicates (Felsenstein 1985) in PAUP* 4.0b, and clade support in BI analyses was evaluated using posterior probabilities.

Tree searches in ML analyses were performed with the starting trees obtained from 100 random stepwise additions followed by tree-bisection-reconnection (TBR) branch swapping. In 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 two independent runs of $10 \times 10^6$ generations in which trees were sampled every $10^3$ generations, resulting in $10^4$ samples for each run. The first $10^3$ trees of each run were discarded as burn-in, and a majority-rule consensus tree was constructed using the final $1.8 \times 10^3$ trees. The analysis was stopped when the average standard deviation of split frequencies approached zero, and convergence also was assessed using Tracer 1.5 (Rambaut and Drummond 2007). The combined data set was analyzed in a partitioned manner to allow independent convergence on optimal values for each fragment (Ronquist and Huelsenbeck 2003).

Each gene fragment was analyzed separately then compared visually to evaluate potential conflict among resulting gene trees. Nodes were considered well supported if there was > 85% bootstrap support or significant Bayesian posterior probabilities > 0.90. Because analyses of
individual data sets showed no conflicting clades that were well supported, all genes were combined and analyzed in a single matrix partitioned by gene.

**Analysis of divergence times.**—The program *BEAST version 1.6.0* (Drummond and Rambaut 2007) was used to estimate the times of divergence among *P. difficilis, P. nasutus, P. truei, and P. attwateri*. Several representative species from clade VI of the tribe Reithrodontomyini (Miller and Engstrom 2008) were included in the *BEAST analysis, as follows: *Habromys ixtlani, Megadontomys thomasi, Neotomodon alstoni, Onychomys arenicola, O. leucogaster, Osgoodomys banderanus, Peromyscus crinitus, P. eremicus, P. levipes, P. maniculatus, P. melanophrys, and P. melanotis* (Appendix 4.2). *Isthmomys pirrensis* and *Reithrodontomys sumichrasti* were included as outgroups. A Yule tree prior was used, implicitly considering the gene tree to represent the species tree, and the HKY+I+G model was selected as the substitution model. A relaxed clock with a lognormal distribution allowing rate variation among sites was used. Chains were run for $10^7$ generations, sampling the parameter every $10^3$ generations. Convergence statistics were checked for effective sample sizes using Tracer version 1.5 (Rambaut and Drummond 2007). Consensus trees were generated from the resulting trees using TreeAnnotator version 1.6.0 (Rambaut and Drummond 2009) after elimination of 25% as burn-in.

Two fossil-based dates were used to calibrate the *BEAST analysis. The first documents the separation of *Onychomys* from the main stock of *Peromyscus* in the early Pliocene (ca. 5.3 to 3.6 mya; Korth 1994). The second sets the minimum age for *P. eremicus* in late Pleistocene (ca. 126,000 to 5,000 years ago; Martin 1968). To account for uncertainty in the fossil-based calibrations, the fossil-based dates were modeled as lognormal distributions rather than point calibrations (Ho and Phillips 2009).
4.3 RESULTS

Phylogenetic analyses.—A total of 1,140 base pairs (bp) of Cytb, 820 bp of 12S, 654 bp of GHR, and 775 bp of IRBP were sequenced, yielding 3,389 bp for use in analyses. Cytb divergence values (Table 4.1) show two well differentiated units within P. difficilis, one distributed in the Sierra Madre Oriental and Mexican Plateau (the subspecies petricola and difficilis, respectively; Fig. 4.1) and the other occupying the eastern end of the TMVB southward into Oaxaca (saxicola, amplus, and felipensis, respectively). These clades show an average Cytb divergence of 6.7%, whereas within-clade divergence is only 0.7% within the northern clade and 1.2% within the southern clade. Average Cytb divergence between P. nasutus and the northern clade of P. difficilis is 6.85%, and divergence between P. nasutus and the southern clade of P. difficilis is 7.87%. Finally, P. attwateri shows an average divergence of 7.9% from the P. nasutus + P. difficilis clade, and P. truei shows an average of 13.2% Cytb divergence from all other taxa included in Table 4.1.

ML and BI analyses of the Cytb sequences yielded very similar trees that showed only minor differences near the tips of the branches. The ML tree (Fig. 4.2; BI tree available on request) showed subdivision of P. difficilis into the same northern and southern clades evident from the distance data (Table 4.1). These two clades were linked strongly with P. nasutus (100% bootstrap support and 1.0 posterior probability), but relationships among these three lineages were unresolved (Fig. 4.2). ML and BI analyses of 12S generated trees that were concordant with the Cytb tree, but with lower branch support values (trees not shown but available on...
Table 4.1. Percent sequence divergence in the cytochrome-

\( b \) gene (Kimura 2-parameter model) among eight taxa of

*Peromyscus*, including the five currently recognized subspecies of *P. difficilis*. Geographic distributions of the two major clades within *P. difficilis* are indicated. TMVB = Trans-Mexico Volcanic Belt.

<table>
<thead>
<tr>
<th></th>
<th>Mexican Plateau and Sierra Madre Oriental</th>
<th>Eastern end of TMVB southward into Oaxaca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. truei</strong></td>
<td>13.2</td>
<td>13.4</td>
</tr>
<tr>
<td><strong>P. attwateri</strong></td>
<td>13.0</td>
<td>13.6</td>
</tr>
<tr>
<td><strong>P. nasutus</strong></td>
<td>12.6</td>
<td>13.1</td>
</tr>
<tr>
<td><strong>P. d. petricola</strong></td>
<td>13.2</td>
<td>6.9</td>
</tr>
<tr>
<td><strong>P. d. difficilis</strong></td>
<td>13.4</td>
<td>7.2</td>
</tr>
<tr>
<td><strong>P. d. saxicola</strong></td>
<td>7.4</td>
<td>8.1</td>
</tr>
<tr>
<td><strong>P. d. amplus</strong></td>
<td>6.8</td>
<td>8.6</td>
</tr>
<tr>
<td><strong>P. d. felipensis</strong></td>
<td>6.7</td>
<td>8.2</td>
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</table>


Fig. 4.2.—Phylogenetic relationships among the 5 subspecies of *P. difficilis* (sensu Durish et al. 2004), *P. nasutus*, and allied taxa based on a maximum likelihood (ML) analysis of cytochrome-*b* sequences. A Bayesian analysis of the same data yielded a tree with identical topology. Locality numbers are indicated for ingroup taxa and refer to mapped localities in Fig. 4.1 and localities listed in Appendix 4.1. Numbers above branches indicate ML bootstrap support, and numbers below branches indicate Bayesian posterior probabilities. The subspecies *amplus*, *felipensis*, and *saxicola*, formerly assigned to *P. difficilis*, are recognized as junior synonyms of *P. felipensis* (Merriam 1898) in this study.
request). Finally, ML and BI analyses of the two nuclear genes yielded trees with well-supported resolution only at the node defining the northern clade of *P. difficilis* and at the node defining the southern clade of *P. difficilis* (trees not shown but available on request).

ML and BI analyses of the combined data set (partitioned by gene) yielded identical and well-resolved trees (Fig. 4.3). Again I observed the 3 clades evident in the *Cytb* analysis (northern *P. difficilis*, southern *P. difficilis*, and *P. nasutus*; Fig. 4.2), but the trichotomy that was unresolved in the *Cytb* analysis is now resolved to show *P. nasutus* sister to the northern clade of *P. difficilis* (90% bootstrap support and 0.94 posterior probability).

**Estimates of divergence time.**—The tree obtained in the *BEAST* analysis (Fig. 4.4) showed the same northern and southern *P. difficilis* clades evident in the *Cytb* (Fig. 4.2) and 4-gene (Fig. 4.3) analyses. However, the *BEAST* tree differed from the other trees in showing *P. nasutus* sister to *P. attwateri*. The Tracer analysis in *BEAST* confirmed a high effective sample size (> 3,000) for all parameters. All mean estimates of divergence time for the *P. attwateri*, *P. difficilis*, and *P. nasutus* clades were < 1.0 mya (Pleistocene). The split between the northern and southern clades of *P. difficilis* was estimated at approximately 0.7 mya.

### 4.4 DISCUSSION

*P. difficilis* is composed of two genetically well-differentiated lineages, one found in the Sierra Madre Oriental and Mexican Plateau (the subspecies *petricola* and *difficilis*, respectively; Fig. 4.1) and the other occupying the eastern end of the TMVB southward into Oaxaca (*saxicola*, *amplus*, and *felipensis*, respectively). This subdivision of *P.*
Fig. 4.3.—Phylogenetic relationships among selected specimens of _P. difficilis_ and _P. nasutus_ based on a partitioned maximum likelihood (ML) analysis of two mitochondrial genes (_Cytb_ and _12S_) and 2 nuclear genes (_GHR_ and _IRBP_). A Bayesian analysis of the same data yielded a tree with identical topology. Locality numbers are indicated for ingroup taxa and refer to mapped localities in Fig. 4.1 and localities listed in Appendix 4.1. Numbers above branches indicate ML bootstrap support, and numbers below branches indicate Bayesian posterior probabilities. The subspecies _amplus_, _felipensis_, and _saxicola_, formerly assigned to _P. difficilis_, are recognized as junior synonyms of _P. felipensis_ (Merriam 1898) in this study. Original subspecies assignments are indicated in parentheses: _a_ = _amplus_, _d_ = _difficilis_, _f_ = _felipensis_, _p_ = _petricola_, _s_ = _saxicola_.

![Phylogenetic tree diagram](image-url)
difficilis into two divergent clades is consistent with the findings of Durish et al. (2004), although they did not include samples of P. d. petricola or P. d. felipensis in their study. The 4-gene partitioned analysis (Fig. 4.3) shows a sister relationship between the northern clade of P. difficilis and P. nasutus, with the southern clade of P. difficilis outside this group.

Specimens from Durango originally identified as P. difficilis by Avise et al. (1979) and Durish et al. (2004) were represented by the specimen from locality 5 in this study (Fig. 4.1). Durish et al. (2004) reported that their specimens from Durango were more closely related to P. nasutus than to P. difficilis and my results concur in showing strong evidence (Fig. 4.2) that these specimens from Durango represent a southward range extension of P. nasutus into the Sierra Madre Occidental of Mexico. This finding also is consistent with the chromosomal findings of Robbins and Baker (1981), who reported a P. nasutus-like karyotype from Peromyscus specimens from Durango. A detailed reassessment of the distributions of P. difficilis and P. nasutus in northern Mexico is needed.

Average Cytb genetic distances between the three major clades of Peromyscus identified in this analysis (P. nasutus and the northern and southern clades of P. difficilis) range from 6.7% to 7.9% (Table 4.1) and compare favorably with Cytb distances measured between sister species of Peromyscus reported in previous studies (5.8–7.7%; Baker and Bradley 2006).

Biogeographical considerations.— Recent distributional, paleontological, and molecular studies of Mexican mammals have shown that most phyletic diversification in
desert-dwelling lineages took place during Miocene and Pliocene times (Hafner and Riddle 1997; Riddle 1995; Riddle et al. 2000; Vrba, 1992). In contrast, phyletic diversification in lineages of mammals inhabiting the highlands of the TMVB, the Sierra Madre Oriental, and the Sierra Madre Occidental seems to have been influenced more strongly by Pleistocene climate cycles (Findley 1969; León-Paniagua et al. 2007; Orr 1960). Because populations of mammals currently recognized as *P. difficilis* inhabit both xeric habitats at mid-elevations and mesic habitats at higher elevations in Mexico, it was impossible to predict prior to this study whether phyletic diversification in *P. difficilis* was influenced more by Pleistocene or pre-Pleistocene climatic and geologic events.

The divergence times estimated in this study (Fig. 4.4) suggest that the two lineages of *P. difficilis* diverged during the Pleistocene. If so, the major mountain chains of northern Mexico (TMVB, Sierra Madre Oriental, and Sierra Madre Occidental), instead of acting as a barrier to range expansion in rodents, may have acted as a dispersal corridor connecting the three mountain ranges during warm interglacial cycles. Repeated expansion and contraction of the geographic range of *P. difficilis* may have resulted in isolation of the southern lineage in the eastern TMVB promoting the genetic differentiation we see today between the northern and southern clades of *P. difficilis*. The same or a similar process has been hypothesized to explain phyletic diversification in other peromyscine taxa of Mexico, including *Reithrodontomys, Habromys*, the *P. aztecs* species group, and the *P. maniculatus* complex (Arellano et al 2005; Avise et al. 1979; Bradley et al. 2004a, 2004b; Dawson 2005; Hibbard 1968; Kalkvik et al. 2011, León-Paniagua et al. 2007; Sullivan et al. 1997, 2000).
Fig. 4.4.—Estimates of divergence times in *P. difficilis*, *P. felipensis*, and close relatives calculated from *BEAST* analysis. Numbers at nodes are mean divergence dates, and bars show 95% credibility intervals. Additional outgroup taxa (listed in the text) were included in the analysis, which used two dated fossils to calibrate the tree.
Taxonomic conclusions.—*P. difficilis* was originally described by Allen (1891) from a specimen taken in Sierra de Valparaíso, Zacatecas. Seven years later, *P. felipensis* was described (Merriam 1898) from specimens collected in Cerro San Felipe, near Oaxaca City, and in 1909, Osgood placed *felipensis* in synonymy under *P. difficilis*. Herein, I return *P. felipensis* to full species status based on phylogenetic analysis of mitochondrial and nuclear genes, estimates of divergence time from its sister lineage (most likely *P. nasutus*), geographic distribution, and morphology. Synonymies of *P. difficilis* and *P. felipensis* follow.

*Peromyscus difficilis* (J. A. Allen, 1891)

Zacatecan deermouse

*Vesperimus difficilis* J. A. Allen, 1891:518. Type locality “Sierra de Valparaíso, Zacatecas, Mexico.”


*P. d. petricola* Hoffmeister and de la Torre, 1959:167–168. Type locality “12 mi. E. San Antonio de las Alazanas, 9000 ft., Coahuila, Mexico.”

**Geographic range.**— *Peromyscus difficilis* is endemic to Mexico and is found throughout the Sierra Madre Oriental and adjacent mountain ranges from southwestern Chihuahua and southeastern Coahuila, southward through the low hills of Durango and Zacatecas. This species probably occurs in the Sierra Madre Occidental as well. Its range continues southward from Zacatecas into parts of San Luis Potosí and mountainous regions of Guanajuato (Fig. 4.1; Fernández et al. 2010; Hall 1981; Hoffmeister and de la Torre 1961; Osgood 1909).
Description.—Size medium to large for Peromyscus; total length 230 mm in adult males (range 223–237 mm); 237 mm in adult females (range 227–250 mm); tail length 128.6–132.5 mm. Fur dull and dark dorsally with little or no ochraceous, and heavily overlaid with black. Auditory bullae greatly inflated; brain case and interorbital region broad; skull, nasals, and toothrow long (Hoffmeister and de la Torre 1961; Fernández et al. 2010).

Comments.—The currently recognized subspecies P. d. difficilis and P. d. petricola were not recognized as monophyletic clades in this analysis (Fig. 4.2), so until additional research on subspecific variation within P. difficilis is completed, no formal subspecies are recognized in this study.

Peromyscus felipensis Merriam, 1898
Southern rock deermouse

P. felipensis Merriam, 1898:122–123. Type locality “Cerro San Felipe, Oaxaca, Mexico (alt. 10,200 ft.).”

P. amplus Osgood, 1904:62–63. Type locality “[San Juan Bautista] Coixtlahuaca, Oaxaca, Mexico.”

P. difficilis saxicola Hoffmeister and de la Torre, 1959:168–169. Type locality “Cadereyta, 2100 meters, Querétaro, México.”

Geographic range.—P. felipensis occurs in Querétaro, northern Hidalgo, the mountains of southern Hidalgo, northern México state, Tlaxcala, Puebla, westcentral Veracruz, and northcentral Oaxaca (Goodwin 1954; Hall 1981; Hoffmeister and de la Torre 1961; Osgood 1904, 1909; Villa-Ramírez 1953; Fig. 4.1).
Description.—Size medium to large for *Peromyscus*; total length of 10 adult topotypes (including both sexes) 241.5 mm (range 225–248 mm); tail length 118–132 mm. Fur glossy and unicolored dorsally; dorsal coloration ochraceous, brownish or reddish in northern populations to blackish in the southernmost populations. Southern populations of *P. felipensis* (the former subspecies *P. d. amplus* and *P. d. felipensis*) have a narrower brain case and interorbital region compared to *P. difficilis*; northern populations (the former *P. d. saxicola*) are smaller in overall size than southern populations (Hoffmeister and de la Torre 1959, 1961; Fernández et al. 2010).

Comments.—The currently recognized subspecies *P. d. felipensis*, *P. d. amplus*, and *P. d. saxicola* were not recognized as monophyletic clades in this analysis (Fig. 4.2), so until additional research on subspecific variation within *P. felipensis* is completed, no formal subspecies are recognized in this study.

4.5 LITERATURE CITED


members of the *truei* and *maniculatus* species groups. Journal of Mammalogy 60:177–192.


CHAPTER 5
MOLECULAR SYSTEMATICS AND BIOGEOGRAPHY OF THE MEXICAN ENDEMIC KANGAROO RAT, *DIPodomys phillipsii* (RODENTIA: HETEROMYIDAE)

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5.1 INTRODUCTION

Phillips’ kangaroo rat (*Dipodomys phillipsii*) is a threatened, endemic rodent of Mexico (Luiselli 2002). Only a few taxonomic studies (all based on morphology) have focused on this species, and the systematic status of the four currently recognized subspecies is unknown. *Dipodomys phillipsii* is a medium sized kangaroo rat (total length 230–304 mm) with four toes on the hind limbs, a relatively short tail with black stripes united at the distal one-third of the tail, and a whitish tuft on the tip of the tail (Hall 1981; Jones and Genoways 1975; Schmidly et al. 1993). This species is distributed along a narrow band of dry, semi-desert habitat extending from southeastern Durango to the Tehuacán–Cuicatlán Valley of southern Puebla and northern Oaxaca (McMahon 1979; Schmidly et al. 1993; Fig. 5.1). The current taxonomy of *D. phillipsii*, which is based on morphometric and qualitative morphological traits, is as follows: *D. p. phillipsii* is found mainly in the Valley of México, in the Distrito Federal, and in the states of México and Hidalgo; *D. p. ornatus* is known from Durango, Zacatecas, Aguascalientes, San Luis
Fig. 5.1.—Map of central Mexico modified from Hall (1981) and Jones and Genoways (1975) showing the geographic distribution, sampling localities, and current subspecies of Phillips’ kangaroo rat, *Dipodomys phillipsii*, in Mexico. Localities are listed in Appendix 5.1.
Potosí, Querétaro, Guanajuato, and Jalisco; *D. p. perotensis* is found in eastern Tlaxcala, central-western Veracruz, and Puebla; and *D. p. oaxacae* is known from southern Puebla and northern Oaxaca (Hall 1981; Jones and Genoways 1975; Wilson and Reeder 2005; Fig. 5.1).

*Dipodomys phillipsii* was described by Gray (1841) based on one specimen from near Real del Monte, Hidalgo. Some 50 years later, Merriam (1894) collected specimens in the northern portion of the species range (Berriozábal, Zacatecas) and in the Oriental Basin (Cuenca Oriental) in central Mexico (Perote, Veracruz) and described *D. ornatus* and *D. perotensis*, respectively. Merriam (1894) emphasized differences in size and fur color between these taxa, and he noted that the skulls were very similar, showing only variation in proportions. Davis (1944) compared individuals of *D. phillipsii* and *D. perotensis* and was unable to see the differences that Merriam (1894) reported. As a result, Davis (1944) suggested that *D. perotensis* be regarded as a subspecies of *D. phillipsii*. Hooper (1947) trapped specimens of *D. phillipsii* in Teotitlán, Oaxaca, and described *D. p. oaxacae* based also on size and fur color, recognizing the cranial similarities between his specimens and those of the geographically nearest subspecies, *D. p. perotensis*.

Genoways and Jones (1971) analyzed specimens from several populations throughout the range of *D. phillipsii* and *D. ornatus* and suggested that a suite of morphological characters, including mastoid breadth, maxillar breadth, interorbital breadth, body size, and pelage coloration could be used to distinguish among the subspecies of *D. phillipsii*. They reported low levels of within-population variation in cranial dimensions, but high levels of within-population variation in pelage coloration.
Because they could find no consistent morphological difference between *D. ornatus* and *D. phillipsii*, they reduced *D. ornatus* to subspecific status under *D. phillipsii*. Genoways and Jones (1971) generated a phenogram based on a morphological distance matrix but found no congruence between the phenogram and the geographic locations of their samples.

Evolutionary relationships among the geographic subunits of *D. phillipsii* have remained controversial since publication of the study by Genoways and Jones (1971). In this study we use mitochondrial and nuclear DNA sequence data to test species monophyly and assess phylogenetic relationships within *D. phillipsii*. To place our results in a larger, historical biogeographical context, we estimate the time of major divergence events within *D. phillipsii* and among its close relatives.

5.2 MATERIALS AND METHODS

**Sampling, amplification, and sequencing.**—Samples of *D. phillipsii* tissue or ear clips were either collected in the field under the authority of the Mexican collecting permit FAUT-0002 issued to FAC or donated to us by museums (Appendix 5.1). We sequenced specimens from 17 localities in northern and central Mexico, including six localities of *D. p. ornatus* (*n* = 7 individuals), one locality of *D. p. phillipsii* (*n* = 1); eight localities of *D. p. perotensis* (*n* = 8), and two localities of *D. p. oaxacae* (*n* = 5; Fig. 5.1; Appendix 5.1). Outgroup sequences for *D. agilis*, *D. californicus*, *D. compactus*, *D. deserti*, *D. elator*, *D. heermanni*, *D. merriami*, *D. microps*, *D. nelsoni*, *D. ordii*, *D. panamintinus*, *D. spectabilis*, and *Heteromys irroratus* (use of *Heteromys* follows the taxonomy of Hafner et al. 2007) were obtained from Genbank or generated for this study (Appendix 5.1). The collection and processing of samples were undertaken following the
Total genomic DNA was extracted from tissue using a standard phenol-chloroform protocol (Darbre 2001; Saunders and Parkes 1999) and a commercial kit (DNEase Blood and Tissue Kit; Qiagen Inc., Valencia, California). Two nuclear and two mitochondrial genes were sequenced. The nuclear genes code for the growth hormone receptor (GHR) and the interphotoreceptor retinoid-binding protein (IRBP), and the mitochondrial genes code for a subunit of the respiratory chain protein Cytochrome-\(b\) (\(Cytb\)) and the 12S ribosomal RNA gene (\(12S\)). Sequences were amplified by PCR (Saiki et al. 1988) using universal primers developed for rodents (Appendix 5.2). The following PCR parameters were used to amplify both mitochondrial genes from fresh tissue samples: initial denaturation at 95°C for 2 min, followed by 27 cycles of denaturation at 95°C for 1 min, annealing at 49°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min (Mantooth et al. 2000). PCR parameters for mitochondrial genes obtained from dry skin clips were: denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR parameters for the nuclear gene GHR were: denaturation at 94°C for 5 min, followed by 34 cycles at 94°C for 15 s, 60°C for 1 min, and 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR amplification of the IRBP gene was performed as follows: denaturation at 95°C for 10 min, followed by 27 cycles at 95°C for 25 s, 58°C for 20 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplifications were performed with 200 ng of DNA in a total volume of 25 µL. Agarose (2%) gels were used to visualize amplified products. PCR products
were purified using a QIAquick PCR Purification Kit (Qiagen Inc.) with either Polyethylene Glycol or ExoSAP-IT (Affymetrix, Santa Clara, California). DNA sequencing was performed for both light and heavy strands with a Big Dye Terminator v1.1, v3.1 in an automated 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) at the Instituto de Biología, Universidad Nacional Autónoma de México and at the Museum of Natural Science, Louisiana State University.

**Distance analysis.**—Editing and alignment of sequences and matrix manipulations were performed in Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Michigan), MacClade (Maddison and Maddison 2000), and Mesquite (Maddison and Maddison 2010). Sequences were verified manually, and authenticity of the gene was confirmed by amino acid translation and BLAST searches in Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

To enable comparison of our results with those of previous studies, average uncorrected sequence divergence values for the *Cytb* gene were corrected using the Kimura 2-parameter substitution model (Kimura 1980) in PAUP* (Phylogenetic Analysis using Parsimony, version 4.0b, Swofford 2003). This model has been used widely in studies of *Cytb* variation in mammals (Bradley and Baker 2001; Honeycutt et al. 1995). We also calculated average maximum and minimum divergence values for each of the main clades in our study. To detect phylogenetic “noise,” saturation analyses for 3rd codon positions were performed as described by Griffiths (1997). To explore the effects of 3rd position substitutions in phylogenetic reconstruction, we ran maximum likelihood (ML) analyses including all 3rd position substitutions, omitting all 3rd position substitutions, and excluding only 3rd position transitions.
Phylogenetic analyses.—Our preliminary phylogenetic analysis included all specimens ($n = 21$ individuals of *D. phillipsii*) for which we had partial *Cytb* sequences (1,022 base pairs, bp). To test monophyly of *D. phillipsii*, we included sequences from the closely related species *D. merriami* ($n = 2$), and *D. elator* ($n = 2$) as well as one sequence from several other species of the genus *Dipodomys* and *Heteromys irroratus* (Appendix 5.1; Alexander and Riddle 2005; Hafner et al. 2007). We selected five individuals from the Trans-Mexico Volcanic Belt (TMVB) clade and five individuals from the Mexican Plateau (MP) clade and sequenced these individuals for portions of the *12S*, *GHR*, and *IRBP* genes. A partition homogeneity test in PAUP* (Swofford 2003; 1,000 replicates, $P = 0.68$) showed no significant heterogeneity among individual data sets, so the mitochondrial and nuclear data sets were concatenated for the remaining analyses, which were carried out with at least five representatives of each major clade. Variable nucleotide positions were considered unordered, discrete characters with four possible states (A, C, G, T). Phylogenetic analyses were carried out under a ML framework in PAUP* or PhyML 3.0 (Guindon and Gascuel 2003). Analyses based on Bayesian inference (BI) were conducted using MrBayes (version 3.2-cvs; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best-fit models for ML and BI analyses were evaluated using the Akaike Information Criterion and the programs jModeltest 0.1.1 (Akaike 1973; Guindon and Gascuel 2003; Posada 2008), MrModeltest (version 2.2; Nylander 2004), or Modeltest (version 3.7; Posada and Buckley 2004; Posada and Crandall 1998). The GTR+$I+G$ model was selected for the mitochondrial sequences, and the HKY+$G$ model was selected for the nuclear sequences. ML clade
support was assessed with 500 bootstrap (Felsenstein 1985) replicates in PAUP*, and clade support in BI analyses was evaluated using posterior probabilities.

Tree searches in the ML analyses were performed with the starting trees obtained via 100 random, stepwise additions followed by tree-bisection-reconnection (TBR) branch swapping. To test preliminary results that showed a paraphyletic *D. phillipsii*, we conducted an additional ML analysis forcing *D. phillipsii* to be monophyletic and compared this tree to the unconstrained tree using the Kishino-Hasegawa test (Kishino and Hasegawa 1989; Schmidt 2009). 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 two independent runs of $10^6$ generations in which trees were sampled every $10^3$ generations, resulting in $10^4$ samples for each run. The first $10^3$ trees of each run were discarded as burn-in, and a majority-rule consensus tree was constructed using the final $1.8 \times 10^3$ trees. The analysis was stopped when the average standard deviation of split frequencies approached zero, and convergence also was assessed using Tracer 1.5 (Rambaut and Drummond 2007). The combined data set was analyzed as partitioned (genes and model parameters) to allow for independent convergence on optimal values for each component (Ronquist and Huelsenbeck 2003).

The data sets were partitioned into mitochondrial versus nuclear genes, *Cytb* versus *12S* genes, and *GHR* versus *IRBP* genes. Whenever possible, each gene fragment was analyzed separately to evaluate potential conflict among gene trees. Nodes were considered well supported if there was > 80% bootstrap support in ML analyses or > 95% posterior probability in BI analyses.
Divergence time analysis.—We used the program *BEAST version 1.6.0 (Drummond and Rambaut 2007) to generate an estimate of the timing of divergence of the basal polytomy involving *D. phillipsii* and its close relatives. The *BEAST* analyses included one *Cytb* sequence for each of the ingroup and outgroup species. A Yule tree prior was used, implicitly considering the gene tree to represent the species tree. The absence of dated fossils of *D. phillipsii* prevented us from using fossil-based time calibrations, so instead we used global substitution rates to calibrate our analyses (Drummond et al. 2007). Substitution rates calculated exclusively for the genus *Dipodomys* are not available in the literature, so we used three published estimates for other rodent species. The first substitution rate estimate was 4.0% per 1.0 x 10^6 years calculated for the split between the pocket gopher genera *Pappogeomys* and *Cratogeomys* (DeWalt et al. 1993). *Pappogeomys* and *Cratogeomys* belong to the family Geomyidae, which is sister to the Heteromyidae. The second rate estimate we used was 4.78% per 1.0 x 10^6 years generated for the more distantly related house mouse *Mus musculus*, and the third was 5.23% per 1.0 x 10^6 years generated for the brown rat *Rattus norvegicus* (Bininda-Emonds 2007). We excluded the frequently used rate of 2% per 1.0 x 10^6 years because it has been shown recently to be an underestimate of actual rates (Nabholz et al. 2009). We used a relaxed clock with a lognormal distribution allowing rate variation among sites. Chains were run for 10^7 generations, sampling the parameter every 10^3 generations. Convergence statistics were checked for effective sample sizes using Tracer version 1.5 (Rambaut and Drummond 2007). Consensus trees were generated from the resulting 10^4 trees using TreeAnnotator version 1.6.0 (Rambaut and Drummond 2009) after elimination of 10% as burn-in.
5.3 Results

Distance and phylogenetic analyses.—We sequenced 1,022 bp of the Cytb gene, 796 bp of the 12S gene, 820 bp of GHR, and 470 bp of IRBP, yielding a total of 3,110 bp for use in the analyses. Initial ML and BI analyses using only Cytb sequences from the 21 individuals generated trees that differed only in relationships among terminal taxa (Fig. 5.2). In these trees, *D. phillipsii* is divided into two well-supported clades, the MP clade, which contains all specimens currently assigned to *D. p. ornatus* (localities 1–6 in Fig. 5.1) and the TMVB clade, which contains all other *D. phillipsii* samples in this analysis (localities 7–17 in Fig. 5.1). Initial analyses grouped the MP clade of *D. phillipsii* with *D. elator*, suggesting paraphyly of *D. phillipsii*, but support for this group was weak, causing us to collapse the two *D. phillipsii* clades plus *D. elator* and *D. merriami* into a strongly supported four-way polytomy (Fig. 5.2). The Kishino-Hasegawa test showed the tree with the MP clade of *D. phillipsii* sister to *D. elator* to be a slightly better fit to the sequence data than the tree forcing *D. phillipsii* to be monophyletic (-lnL = 3529.6743 and 3673.9037, respectively), but the difference was not statistically significant (*P* > 0.285; 1-tailed test).

The analysis of substitutional saturation showed Cytb 3rd position transitions to be saturated, so ML analyses were re-run twice, once excluding all 3rd position substitutions and again excluding only 3rd position transitions. Trees resulting from both analyses showed the same two clades of *D. phillipsii* (as in Fig. 5.22) but, strong bootstrap support for relationships within the *D. phillipsii + D. elator + D. merriami* clade was lacking.
Representatives of the *D. phillipsii* TMVB clade (five individuals from four localities), *D. phillipsii* MP clade (five individuals from three localities), and two representatives each of *D.*

Fig. 5.2.—Phylogram showing placement of Phillips’ kangaroo rat, *D. phillipsii*, within the genus *Dipodomys* based on a maximum likelihood analysis of Cytochrome-*b* sequences. Numbers before state names refer to localities mapped in Fig. 5.1. The gray dot corresponds to the divergence time of approximately 4 mya estimated using *Beast*. Numbers above branches are Bayesian posterior probabilities and numbers below branches are ML bootstrap support values. Black dots represent nodes with Bayesian posterior probabilities ≥ 0.95 and ML bootstrap values ≥ 85. A Bayesian analysis of the same data yielded a tree that differed only in minor rearrangements of the terminal branches.
*D. elator* and *D. merriami* were sequenced for the other three genes (*12S, GHR,* and *IRBP*). ML and BI analyses of the *12S* sequences showed basically the same tree as in Fig. 5.2, with only minor differences near the tips of the branches (tree not shown). Separate analyses of the nuclear genes showed little or no resolution at the shallow nodes but recovered the same major clades seen in the analyses of the mitochondrial genes (Fig. 5.2). The partition homogeneity test showed no significant heterogeneity among individual data sets, so the mitochondrial and nuclear sequences were concatenated for all remaining analyses.

Bayesian analyses of the concatenated data set using the data partitions defined earlier showed the same subdivision of *D. phillipsii* into two well-defined clades (MP and TMVB) and the same four-way polytomy involving the two clades of *D. phillipsii, D. elator,* and *D. merriami* (Fig. 5.3). As in the other analyses, branch support values were generally higher for the basal nodes and slightly lower for some of the shallower nodes in the trees.

*Cytb* divergence values (Table 5.1) show the two lineages of *D. phillipsii* (MP and TMVB) to be approximately 9.8% genetically divergent. In contrast, average within-clade divergence was only 1.5% for the MP clade and 1.2% for the TMVB clade. Genetic distances between the two *D. phillipsii* clades and *D. elator* ranged from 11.3% to 11.4% and distances between the two *D. phillipsii* clades and *D. merriami* ranged from 11.6% to 12.3%.

**Estimates of divergence time.**— Except for minor rearrangement of terminal taxa, the tree obtained in the *BEAST* analysis was identical to those generated in the ML and
Fig. 5.3.—Phylogram showing placement of Phillips’ kangaroo rat, *D. phillipsii*, within allied species of *Dipodomys* based on a partitioned maximum likelihood (ML) analysis of two mitochondrial genes (*Cytb* and *12S*) and two nuclear genes (*GHR* and *IRBP*). Numbers before state names refer to localities mapped in Fig. 5.1. Numbers above branches are Bayesian posterior probabilities and numbers below branches are ML bootstrap support values. A Bayesian analysis of the same data yielded a tree with identical topology.
Table 5.1.—Mean percent cytochrome-\(b\) sequence divergence values (Kimura 2-parameter model) and ranges (in parentheses) between and within the two major clades of *Dipodomys phillipsii* (Trans-Mexico Volcanic Belt, \(n = 14\); Mexican Plateau, \(n = 7\)), *D. elator* \((n = 2)\), and *D. merriami* \((n = 2)\).

<table>
<thead>
<tr>
<th></th>
<th>Trans-Mexico Volcanic Belt</th>
<th>Mexican Plateau</th>
<th><em>D. elator</em></th>
<th><em>D. merriami</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trans-Mexico Volcanic Belt</strong></td>
<td>1.2 (0.7–2.2)</td>
<td>9.8 (9.2–10.2)</td>
<td>11.4 (10.8–12.0)</td>
<td>12.3 (12.0–12.8)</td>
</tr>
<tr>
<td><strong>Mexican Plateau</strong></td>
<td></td>
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<tr>
<td><strong>D. elator</strong></td>
<td></td>
<td></td>
<td></td>
<td>11.6 (11.1–12.6)</td>
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<tr>
<td><strong>D. merriami</strong></td>
<td></td>
<td></td>
<td></td>
<td>12.7</td>
</tr>
</tbody>
</table>
BI analyses (Fig. 5.2). The Tracer analysis in *BEAST confirmed a high effective sample size (> 3,000) for all parameters. Estimates of divergence time (95% highest posterior density interval) for the node that marks the separation of the two *D. phillipsii clades, *D. elator, and *D. merriami (gray dot in Fig. 5.2) were 2.86–6.46 mya when the *Pappogeomys–*Cratogeomys rate calibration was used, 2.74–6.16 mya when the *Mus musculus* calibration was used, and 2.83–6.24 mya when the *Rattus norvegicus* calibration was used. The mean node age based on these three estimates ranged from 4.19 to 4.29 mya.

### 5.4 Discussion

All phylogenetic analyses (ML, BI, and *BEAST) of the concatenated sequence data recovered a strongly supported clade composed of four genetically well-differentiated lineages: *D. phillipsii* (TMVB), *D. phillipsii* (MP), *D. elator*, and *D. merriami* (Fig. 5.3). The composition of this clade is consistent with the molecular findings of Mantooth et al. (2000) and Hafner et al. (2007), although neither of those studies included a sample of *D. phillipsii* from the TMVB and Hafner et al. (2007) did not include a sample of *D. elator*. Our trees (Figs. 5.2 and 5.3) show *D. ordii* and *D. compactus* to be sister to the above-mentioned clade, which is consistent with the findings of Mantooth et al. (2000; although that study did not include *D. compactus*) and does not conflict with the findings of Hafner et al. (2007) if 1 branch (with questionable support in that study: maximum parsimony bootstrap < 75%, ML bootstrap = 77%, and BI posterior probability = 1.0) that lead to *D. ordii* and *D. compactus* as sister to a clade formed by *D. panamintinus*, *D. heermanni*, *D. agilis*, *D. microps*, and *D. californicus* is collapsed.
Although phyletic resolution within the *phillipsii + elator + merriami* clade was poor, certain of our analyses suggested (with only weak branch support) that the two *D. phillipsii* clades may not be sister lineages. In contrast, the smallest *Cytb* genetic distance between clades in Table 5.1 (9.8%) was between the two *D. phillipsii* clades, thus supporting their sister status, followed by *D. phillipsii* (MP clade) and *D. elator* (11.3%). *Cytb* distance calculations should be viewed with caution, however, because it is well known that distance values can be misleading when substitution rates are heterogeneous among lineages (Rzhetsky and Sitnikova 1996), and our test for homogeneous substitution rates in our dataset led us to reject the assumption of clock-like behavior (PAUP*; *P* = 0.0001). It also is important to note that accuracy of distance values lessen when they exceed 10% (Jin and Nei 1990), which is the case in most of our inter-clade comparisons in Table 5.1.

Although our results are ambiguous with respect to the question of *D. phillipsii* monophyly, this question may be moot considering that the two lineages within what is now considered *D. phillipsii* (MP and TMVB) approach 10% *Cytb* sequence divergence. According to our estimates, divergence of these two lineages occurred approximately 4 mya, roughly contemporaneous with divergence of *D. merriami* and *D. elator*. We were unable to resolve this four-way polytomy despite using a large number of base pairs from multiple genes (both mitochondrial and nuclear) and multiple phylogenetic approaches. We interpret our inability to resolve the branching sequence in this clade as resulting from near-simultaneous and rapid divergence of these four clades such that few, if any, molecular changes that would reveal the order in which these taxa diverged remain to be discovered today (Lanyon 1988; Spradling et al. 2004).
Even if future studies show the two clades of *D. phillipsii* to be sister lineages, this finding would be irrelevant to the question of whether these lineages are genetically isolated and may represent distinct species. Although we are reluctant to base species-level designations primarily on level of *Cytb* divergence, the level of *Cytb* sequence divergence between these lineages (9.8%) is not trivial—it is well above the mean level of divergence (7.3%) measured between 19 pairs of sister species of rodents reported by Baker and Bradley (2006) in their discussion of the genetic species concept.

**Biogeographical considerations.**—It has been proposed that the North American mammalian biota is young by geological standards and that most species-level diversification within mammals was largely in response to glacial and interglacial changes during the Pleistocene (Findley 1969; Orr 1960; Schmidly et al. 1993). However, new distributional, paleontological, and molecular evidence has shown that most phyletic diversification of the mammals of North American deserts took place during Miocene and Pliocene times (Hafner and Riddle 1997; Riddle 1995; Riddle et al. 2000; Vrba, 1992). Our time estimates suggest that diversification of the two lineages of *D. phillipsii* also occurred prior to the major climatic fluctuations of the Pleistocene, and we contend that the Trans-Mexico Volcanic Belt, which is widely recognized as a generator of biological diversity, may have played a causal role in this divergence. The long and slow rise of this complex of mountain ranges that extends nearly from the Pacific ocean to the Gulf of Mexico has been implicated as a causal force in phyletic diversification at multiple taxonomic levels in many organisms, frequently resulting in genetically isolated lineages north and south of the TMVB (Devitt 2006; Douglas et al. 2010; Esteva et al. 2010; Mateos et al. 2002; McCormack et al. 2008; Mulcahy and
Mendelson 2000; Sosa et al. 2009; Zink and Blackwell 1998). We believe that the two lineages of *D. phillipsii* are yet another example of the powerful isolating force of the TMVB.

Researchers have proposed multiple hypotheses for diversification of *D. elator*, *D. merriami*, and *D. phillipsii*, most assuming that this occurred in the Mexican Plateau and northward (Alexander and Riddle 2005; Lidicker 1960; Mantooth et al. 2000; Morafka 1977; Savage 1960). Most of these scenarios suggest that diversification took place in response to development of extensive desert areas in North America, coincident with the uplifting of mountains in the United States and Mexico during middle and late Tertiary (Ferrari et al. 2000; Ferrusquia 1998). One hypothesis (Lidicker 1960) suggests that *D. merriami* originally had a wider distribution than it does today and by peripheral isolation gave rise to *D. elator* in Oklahoma and Texas and to *D. phillipsii* in Mexico. Another hypothesis (Mantooth et al. 2000) suggests that a *D. merriami*-like ancestor gave rise to *D. phillipsii*, which originally had a wider distribution than it does today and eventually gave rise to *D. elator* in the southwestern United States. A third scenario (also proposed by Mantooth et al. 2000) suggests that the *D. merriami*-like ancestor first gave rise to *D. elator*, then *D. elator*, in turn, gave rise to *D. phillipsii*. Because our analysis could not resolve the sequence of branching events in this clade, our findings cannot contribute to this debate. However, our findings suggest that each of these hypotheses is missing an important phyletic event: subdivision of ancestral *D. phillipsii* into two distinct clades (the MP and TMVB clades), probably in response to the final uplift of the TMVB in central Mexico.
Taxonomic conclusions.—The Mexican Plateau clade of *D. phillipsii* (currently recognized as *D. p. ornatus*) was originally described as a distinct species, *D. ornatus*, by Merriam (1894). Genoways and Jones (1971) synonymized *D. ornatus* under *D. phillipsii* for lack of consistent morphological differences between the two forms. If *D. ornatus* and *D. phillipsii* are, in fact, genetically isolated species, then the evidence presented by Genoways and Jones (1971) only confirms that they are cryptic, and possibly even sibling, species. Today, populations of the MP clade (*D. p. ornatus*) are separated from populations of the TMVB clade of *D. phillipsii* by > 130 km, and the likelihood that these clades come into contact, much less interbreed, is extremely low. Moreover, the Cytb evidence suggests that these populations have been genetically isolated for approximately four million years. We believe that the sum total of the evidence suggests that *D. ornatus* should again be recognized as a full species distinct from *D. phillipsii*, and below we present a key that can be used to distinguish between these morphologically cryptic species with reasonable (77.4%) accuracy.

*Dipodomys ornatus* Merriam, 1894
Plateau Kangaroo Rat

*Dipodomys ornatus* Merriam, 1894:110. Type locality “Berriozábal, Zacatecas.”

Geographic range.—This species is known only from the Mexican Plateau, where it has been collected in the states of Aguascalientes, Durango, Guanajuato, Jalisco, Querétaro, San Luis Potosí, and Zacatecas.

Description.—Total body length 252–302 mm; 4 toes on hind foot; dorsal coloration pale; interorbital region narrow (57.9–60.9% of mastoid breadth; Genoways
and Jones 1971); mastoid breadth of skull narrow relative to maxillary breadth (ratio between mastoid breadth and maxillary breadth ≤ 1.08).

*Dipodomys phillipsii* Gray, 1841

Phillips’ Kangaroo Rat

(Synonymy under subspecies)

**Description.**—Total body length 230–304 mm; four toes on hind foot; dorsal coloration variable. Populations in the Valley of Mexico (*D. p. phillipsii*) show dark dorsal coloration, medium body size for the species, and broad maxillary and interorbital regions relative to mastoid breadth (Genoways and Jones 1971). Populations in the south (*D. p. oaxaceae*) are small for the species (hind foot < 37 mm), with pale dorsal coloration and narrow maxillary and interorbital breadths relative to mastoid breadth. Populations in the Oriental Basin (*D. p. perotensis*) are slightly darker and larger in body size (hind foot > 37 mm) than *D. p. oaxaceae* and have a somewhat longer cranium than *D. p. phillipsii*.

**Geographic range.**—Known from the arid, semi-desert regions of southwestern Hidalgo, Mexico, Distrito Federal, Tlaxcala, west-central Veracruz, Puebla, and northern Oaxaca.

*D. phillipsii oaxaceae* Hooper, 1947

*D. phillipsii oaxaceae* Hooper, 1947:48. Type locality “Teotitlán, 950 m, Oaxaca.”

**Geographic range.**—Known only from extreme southern Puebla and adjacent northern Oaxaca.

*D. phillipsii perotensis* Merriam, 1894

*D. perotensis* Merriam, 1894:111. Type locality “Perote, Vera Cruz [Veracruz].”
Geographic range.—Known only from the Oriental Basin, west-central Veracruz (vicinity of Perote) and adjacent parts of Puebla and Tlaxcala.

D. phillipsii phillipsii Gray, 1841

Dipodomys phillipii [sic] Gray, 1841:522. Type locality "Mexico, near Real del Monte."

Type locality determined to be in the state of Hidalgo, “in the mountains at the extreme north end of the Valley of Mexico, about 50 miles northeast of the City of Mexico” by Merriam (1893:91). Species name spelled phillipii by typographical error corrected to phillipsii by same author a few months later (Gray 1842. American Journal of Science 42:335).

Macrocetus halticus A. Wagner, 1846:172. Type locality “Mexico.”

Distribution.—Known only from the Valley of Mexico and immediately adjacent areas of Hidalgo, Mexico, and the Distrito Federal.

Key to the species and subspecies of D. phillipsii and D. ornatus

1 Hind foot length ≤ 37.0………………………………………………………………D. p. oaxacae

Hind foot length > 37.0. ………………………………………………………………………2

2 Middorsal pelage very dark (red reflectance < 13%) and specimen taken from vicinity of the Valley of Mexico (Hidalgo, State of Mexico, or Distrito Federal)…D. p. phillipsii

Middorsal pelage very dark to light, but if very dark (red reflectance < 13%) then specimen not taken from vicinity of the Valley of Mexico …………………………... 3

3 Ratio between mastoid breadth of skull and maxillary breadth of skull (mastoid breadth divided by maxillary breadth) > 1.08………………………… D. p. perotensis

*Ratio between mastoid breadth and maxillary breadth ≤ 1.08 ……… D. ornatus
*This character is 77.4% reliable based on examination of 31 randomly selected adult specimens of D. phillipsii perotensis (n = 17) and D. ornatus (n = 14).

5.5 LITERATURE CITED


Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio.


of the California gnatcatcher (*Poliptila californica*). Molecular Phylogenetics and Evolution 9:26–32.
CHAPTER 6

CONCLUSIONS

A vast number of biological studies have focused on the Mexican biota, and at first glance, one’s impression might be that several of the major biological questions have been addressed. However, a careful eye will detect that many species in many parts of the country have yet to be studied, and large numbers of taxa that have been studied previously should be reexamined using modern techniques and analyses. In studies of the Mexican biota, one cannot help but assume that the dramatic climate cycles of the Pleistocene epoch and the prominence of the Trans-Mexico Volcanic Belt (TMVB) played major roles in the origin and diversification of Mexican species. However, only recently have studies been designed to clarify and test the role of Pleistocene climate cycles and the TMVB in generating the biological diversity we see today.

The four nearly codistributed rodent species studied in this dissertation offer excellent opportunities to investigate individual and shared patterns of evolutionary diversification. In each case, I have generated a phylogenetic hypothesis for the taxon, recommended appropriate taxonomic changes, as necessary, and placed the phylogeny in a temporal framework to identify events that may have generated the phylogenetic pattern.

Two of the study taxa, Nelson’s woodrat *Neotoma nelsoni* (Chapter 2) and the Perote ground squirrel *Xerospermophilus perotensis* (Chapter 3), are both endemics to the Oriental Basin in east-central Mexico. Historically, both taxa have been treated as valid species, based mainly on a few qualitative morphological characters and the fact that they are geographically isolated from congenic populations. Previous studies have
postulated that the sister species of *N. nelsoni* and *X. perotensis* occur in the Mexican Plateau and that current populations of these taxa are remnants of once more widespread desert-adapted forms whose distribution was fragmented by Pleistocene climatic oscillations. It is thought that these taxa evolved in isolation in the Oriental Basin following extinction of intermediate populations.

My analyses confirmed that the closest relatives of *N. nelsoni* and *X. perotensis* occur in the Mexican Plateau (*N. leucodon* and *X. spilosoma*, respectively), and my findings also confirmed that *N. nelsoni* and *X. perotensis* are genetically well-differentiated from their sister taxa to the north. However, the Cytb genetic distances (3.3% between *N. nelsoni* and *N. leucodon* and 3.6% between *X. perotensis* and *X. spilosoma*; Table 6.1) are not large, and this in combination with low levels of morphological differentiation between the Oriental Basin and Mexican Plateau populations of these taxa suggest that they should be recognized only at the subspecific level as *N. leucodon nelsoni* and *X. spilosoma perotensis*.

Molecular estimates of divergence times suggested that *N. l. nelsoni* and *X. s. perotensis* diverged from their sister taxa to the north during early Pleistocene times; Table 6.1). Thus, climatic fluctuations during the Pleistocene may have played a role in the early diversification of these lineages, causing repeated fragmentation of their geographic distributions followed by extinction of geographically intermediate populations. Because divergence of *N. l. nelsoni* and *X. s. perotensis* from their relatives to the north following the Miocene/Pliocene uplift of the TMVB, it appears that the TMVB played only a minor, if any, role in the diversification of these lineages. In the
Xerospermophilus study (Chapter 3), a well-differentiated lineage of X. spilosoma was discovered in the Bolsón de Mapimi in Durango, México.

The rock mouse Peromyscus difficilis (Chapter 4) is another Mexican endemic found in the dry foothills of the Sierra Madre Oriental, the Mexican Plateau, the low dry hills of the TMVB, and some apparently disjunct populations in pine forests of central México as far south as Oaxaca. Historically, P. difficilis has been divided into five subspecies based primarily on distribution, but also on a few qualitative morphological characters. The results of my analyses divided this species into two well-supported clades (ca. 6.7% Cytb divergence; Table 6.1)), a northern clade including the subspecies P. d. difficilis and P. d. petricola, and a southern clade containing the subspecies amplus, felipensis, and saxicola. Molecular-based estimates of divergence times suggested that separation of these clades occurred in the Pleistocene, again (as in Chapters 2 and 3) suggesting that glacial and interglacial cycles of the Pleistocene may have influenced genetic differentiation in the common ancestors of these lineages. The southernmost subspecies of P. difficilis (P. d. felipensis) was originally described as P. felipensis. Several lines of evidence, including phylogenetic analyses of mitochondrial and nuclear genes, estimates of divergence times, disjunct geographic distributions, and morphological differences supported my decision to return the southern clade of P. difficilis to full species status as P. felipensis.

My study of the endangered Phillips’ kangaroo rat, Dipodomys phillipsii (Chapter 5), revealed a biogeographic pattern different for that seen in Chapters 2–4. D. phillipsii occurs on the Mexican Plateau and in the arid lowlands of the TMVB. Past studies divided this taxon into several subspecies based mainly on geographic distribution, but
also supported by several qualitative morphological characters. In my analysis, *D. phillipsii* was divided into two well-supported clades (ca. 9.8% *Cytb* divergence; Table 6.1), one distributed on the Mexican Plateau (formerly recognized as *D. ornatus*), and a southern clade in the TMVB. Several lines of evidence, including phylogenetic analyses of mitochondrial and nuclear genes, estimates of divergence times, disjunct geographic distributions, and morphological differences supported my decision to return the Mexican Plateau clade of *D. phillipsii* to full species status as *D. ornatus*. My study showed that *D. phillipsii, D. ornatus, D. elator*, and *D. merriami* form a well-supported clade of kangaroo rats, but I was unable to resolve relationships among these four species. My inability to resolve this polytomy using a large number of base pairs from a combination of mitochondrial and nuclear genes analyzed in multiple ways suggests that diversification among these four species may have occurred over a relatively short period of time.

My molecular-based analyses of divergence times suggests that *D. phillipsii, D. ornatus, D. elator*, and *D. merriami* diverged in mid-Pliocene times; Table 6.1), probably in or near the Mexican Plateau. Unlike the Pleistocene divergence dates reported in Chapters 2–4, this Pliocene divergence suggests that the morphotectonic processes that gave rise to the Trans-Mexico Volcanic Belt may have influenced early diversification in Mexican species of *Dipodomys*.

Many studies of the mammals of Mexico have focused on the rich fauna of the tropical and semitropical regions of southern Mexico. To some extent, the desert environments of Mexico have been ignored, especially by researchers using modern systematic techniques and methods of analysis. By means of this dissertation, I wish to
reaffirm and highlight the importance of Mexico’s deserts, especially the southern extension of the deserts into central Mexico, as natural laboratories and important centers of evolutionary diversification. I invite my colleagues to test the phylogenetic and biogeographic hypotheses contained in this dissertation with their own studies of the flora and fauna of central Mexico.
Table 6.1.—Overview of taxonomic conclusion, divergence values between clades of interest, and mean estimates of divergence time for clades of interest (in million years ago).

<table>
<thead>
<tr>
<th>Species</th>
<th>Monophyly</th>
<th>Divergence values between clades (%)</th>
<th>Mean estimated divergence time (mya)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neotoma nelsoni</td>
<td>Yes</td>
<td>3.2</td>
<td>2.10 Pleistocene</td>
</tr>
<tr>
<td>Xerospermophilus perotensis</td>
<td>Yes</td>
<td>3.6</td>
<td>0.75 Pleistocene</td>
</tr>
<tr>
<td>Peromyscus difficilis</td>
<td>Yes</td>
<td>6.7</td>
<td>0.80 Pleistocene</td>
</tr>
<tr>
<td>Dipodomys phillipsii</td>
<td>Yes</td>
<td>9.8</td>
<td>4.20 Pliocene</td>
</tr>
</tbody>
</table>
APPENDIX 3.1

Specimens used in the analysis of X. s. perotensis relationships listed alphabetically by taxon, locality, geographic coordinates, elevation, catalogue number, sequenced genes, and GenBank numbers. Specimens are housed in the following museums: Colección Nacional de Mamíferos, 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), Los Angeles County Museum of Natural History (LACM), Louisiana State University Museum of Natural Science (LSUMZ), Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC); New Mexico Museum of Natural History (NMMNH), and University of New Mexico Museum of Southwestern Biology (MSB). Numbers in parentheses before localities indicate localities mapped in Fig. 3.1.

*Callospermophilus lateralis*

Collection locality not available. LACM 85487, 12S = AY227530, IRBP = AY227586.

*Cynomys gunnisoni*

United States: (1) 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: (2) Utah, Uintah Co., 8 km E Jensen on Highway 40, CU 1 (EY 1138), 40.369, -109.240, 1,689 m, Cytb = AF157838; 12S = AY227528, IRBP = AY227584. Utah, Uintah Co., 11 km NW Bonanza on Hwy 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* = AY227584.

**Cynomys mexicanus**

Mexico: (3) Nuevo León, Ejido El Tokio, 18 km E highway 57 on highway 58, 24.6, -100.2, 1,891 m, CU 101 (EY 1180), *Cytb* = AF157841; CU 102 (EY 1181), *Cytb* = AF157842.

**Cynomys parvidens**

United States: (4) 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 m from boundary, 37.58, -112.18, 2,466 m, CU 81 (BC1), *Cytb* = AF157929.

**Ictidomys mexicanus mexicanus**

Mexico: (5) México, Parque Nacional Zoquiapan, 15 km SW Rio Frío, 19.3, -98.6, 2,980 m, CU 108 (EY 1210), *Cytb* = AF157848.

**Ictidomys parvidens**

United States: (6) New Mexico, Chaves Co., Eastern NM University, Roswell, West Wells & University Street, 33.39, -104.52, 1,097 m, MSB 135244, *Cytb* = sequences submitted to GenBank, *12S* = sequences submitted to GenBank, *GHR* = sequences submitted to GenBank, *IRBP* = sequences submitted to GenBank; Mexico: (7) Nuevo León, 3 km NE Apodaca, ca. 23 km NE Monterrey, 25.80, -100.16, 408 m, CU 111 (EY 1197), *Cytb* = AF157852; CU 112 (MVA 105), *Cytb* = AF157853.

**Ictidomys tridecemlineatus**

\textit{Xerospermophilus mohavensis}


\textit{Xerospermophilus perotensis}


\textit{Xerospermophilus spilosoma cabrerai}


\textit{Xerospermophilus spilosoma marginatus}


\textit{Xerospermophilus spilosoma pallescens}
Mexico: (15) Durango, 4 km E Ceballos, 26.523, -104.089, 1,117 m, CU 105 (EY 1195),

\( Cytb = \text{AF157845} \); Durango, Ejido La Flor, 20 km E Ceballos, 26.524, -103.929, 1,146 m, CU 106 (EY 1193), \( Cytb = \text{AF157846} \).

\textit{Xerospermophilus tereticaudus neglectus}

United States: (16) Arizona, Pima Co., Tucson, 32.221, -110.926, 917 m, MSB 86022,

\( Cytb = b = \) sequences submitted to GenBank, \( 12S = b = \) sequences submitted to GenBank, \( GHR = b = \) sequences submitted to GenBank, \( IRBP = b = \) sequences submitted to GenBank; MSB 92638, \( Cytb = b = \) sequences submitted to GenBank, \( 12S = b = \) sequences submitted to GenBank, \( GHR = b = \) sequences submitted to GenBank, \( IRBP = b = \) sequences submitted to GenBank; Arizona, Pima Co., 18 km W Tucson, Ryan Field, 32.223, -111.116, 735 m, CU 91 (EY 1169), \( Cytb = \text{AF157940} \); CU 92 (EY 1167), \( Cytb = \text{AF157941} \).

\textit{Poliocitellus franklinii}

United States: Nebraska, Omaha Zoo CU 42 (1A), \( Cytb = \text{AF157893} \); CU 43 (2A), \( Cytb = \text{AF157894} \).

\textit{Urocitellus townsendii idahoensis}

Collection locality not available, CU 86 (EY 1062), \( Cytb = \text{AF157949} \).

\textit{Urocitellus townsendii}

Collection locality not available, CU 87 (EY 1064), \( Cytb = \text{AF157938} \).
APPENDIX 4.1

Specimens examined in the molecular analysis of the rock mouse *Peromyscus difficilis* listed by taxon, state, collecting locality, geographic coordinates, elevation, catalogue number, and GenBank numbers listed in the following order: *Cytb*, *12S*, *GHR*, and *IRBP*. A dash (–) indicates that a specimen was not sequenced for that gene.

Mammal collections where voucher specimens are housed are CWK = C. William Kilpatrick; GK=Ira I. Greenbaum; TK=K. Nutt and Texas Tech University Tissue Number. Carnegie Museum of Natural History (CM); Colección Regional Durango, Centro Interdisciplinario de Investigacion para el Desarrollo Integral Regional-Durango, Instituto Politécnico Nacional (CDR); Louisiana State University Museum of Natural Science (LSUMZ); Museum of Southwestern Biology, University of New Mexico (MSB); Texas A&M University, Texas Cooperative Wildlife Collection (TCWC); Texas Tech University (TTU); Universidad Nacional Autónoma de México, Instituto de Biología (CNMA); Universidad Nacional Autónoma de México, Museo de Zoología “Alfonso L. Herrera”(MZFC); University of Vermont, Zadock Thompson Natural History Collections (ZTNH);. Numbers in parentheses indicate localities mapped in Fig. 4.1. All newly generated sequences were submitted to genbank.

*P. d. amplus*

PUEBLA: (13) 8 mi. SE Chignahuapan, 19.753, -97.947, 8,808 ft., (ZTNHC: CWK 2770) AY376414 *Cytb* only; (12) 3 km S Ciudad Serdan, crossroad between Ciudad Serdan-Esperanza, towards Santa Catarina, 18.932, -97.421, 2,536 m, CNMA 44007 *Cytb*, –, *GHR*, *IRBP*; (12) 1 km S Coyotepec, 19.009, -97.555, 2,435 m, CNMA 44001 *Cytb*, –, *GHR*, *IRBP* (12) 2 km W Guadalupe Victoria, 19.280, -97.378, 2,406 m., CNMA
43992 *Cytb, 12S, GHR, IRBP*; (11) 1.5 km S Oriental, 19.352, -97.635, 2,360 m, CNMA 43966 *Cytb* only. TLAXCALA: (13) 18 km N, 9 km E Apizaco, 19.58, -98.051, 9,142 ft., CM55804, AY387488 *Cytb* only; (12) 2.5 km NW El Carmen Tequexquitla, 19.35, -97.665, 2,378 m, CNMA 43957 *Cytb, 12S, GHR, IRBP*; CNMA 43960 *Cytb, 12S, GHR, IRBP*; (13) Barranca Huehuetitla, 2 km NE San Ambrosio Texantla, 19.316, -98.25, 2,272 m, CNMA 44269 *Cytb* only; (13) Mt. Malinche, 19.265, -98.022, 10,709 ft., TCWC: GK 3904) AY376415 *Cytb* only; (13) 2 km NE Tepetitla, 19.277, -98.365, 7,293 ft., TTU 82690, AY376416 *Cytb* only. VERACRUZ: (10) 3 km S El Frijol Colorado, 19.572, -97.383, 2,435 m, CNMA 43978 *Cytb, 12S, GHR, IRBP*.

**P. d. difficilis**

AGUASCALIENTES: (7) 6 mi. W Rincon de Romos, 22.229, -102.413, 6,852 ft., (TCWC GK:4129) AY376418 *Cytb* only; DURANGO: (5) 50 km W Las Herreras, 25.185, -106.036, 8,526 ft., CRD 1143, AY376417 *Cytb* only; ZACATECAS: (6) 12.4 mi. NW, 16.2 mi. NE (by road) Sombrerete, 23.9, -103.5, 2,268 m, MSB 54457 *Cytb, 12S, GHR, IRBP*; (6) 13.2 mi. NW, 5.4 mi. NE (by road) Sombrerete, 23.9, -103.5, 2,214 m, MSB 55604 *Cytb, 12S, IRBP*; (6) 12.4 mi. NW, 6.2 mi. NE Sombrerete, 23.9, -103.5, 2,010 m, MSB 55616 *Cytb, 12S, IRBP*; MSB 57701 *Cytb, 12S, IRBP*.

**P. d. felipensis**

MEXICO: (15) Highway Ocuilan de Artega-Cuernavaca, km 14, 18.978, -99.416, 7,711 ft., MZFC 5689 *Cytb, 12S, GHR, IRBP*; (14) National Park Izta-Popo-Zoquian, 7.3 km SE Amecameca, 19.1014, -98.6955, 2,848 m, CNMA 45643, *Cytb* only; CNMA 45644 *Cytb, 12S, GHR, IRBP*.

**P. d. petricola**
COAHUILA: (3) 10 mi. E San Antonio de las Alazanas, 25.266, -100.766, 2,586 m, MSB 48201 Cytb only; (3) 13.9 mi. W San Antonio de las Alazanas, 25.266, -100.766, 2,028 m, MSB 48177 Cytb, 12S, GHR, IRBP; NUEVO LEON: (4), Cerro Potosi, Municipio Galeana, 24.85, -100.316, 1,655 m, CNMA 44276, Cyb, –, GHR, IRBP; CNMA 44277 –, 12S, GHR, IRBP; M8647 Cytb, –, –, IRBP; M8667 –, 12S, GHR, P. d. saxicola

HIDALGO: (8) 5.4 mi. SE, 3.2 mi. S Ixmiquilpan, 20.391, -99.316, 6,431 ft., (TCWC GK2642) AF155394 Cytb only; (9) 1.8 mi. E Jonacapa, 20.436, -99.506, 7,480 ft., (TCWC GK3076) AY376419 Cytb only. MEXICO: (11) Cerro Gordo, Santiago Tolman, 19.743, 98.628, 2,500 m, CNMA 43035 Cytb, 12S, GHR, IRBP.

P. nasutus

NEW MEXICO: (1) Socorro Co., Sevilleta, Sepulta Canyon, 34.304, -106.613, 6,470 ft.; MSB 63666 Cytb, 12S, –, –; Cibola Co., 11 mi. S, 14 mi. W San Rafael, 34.963, -108.128, 6,450 ft.; MSB 54841, Cytb, 12S, GHR, IRBP; MSB 54819, Cytb, 12S, GHR, IRBP.

P. nasutus griseus

NEW MEXICO: (1) Lincoln Co., 4 mi. S Carrizozo, 33.583, -105.876, 5,965 ft., TTU 78401, AY155399 Cytb only.

P. nasutus nasutus

TEXAS: (2) Jeff Davis Co., Mt. Livermore Preserve, 30.750, -104.193 5,722 ft., TTU 78316, AY376426 Cytb only.
Neotoma nelsoni

VERACRUZ: (10) 3 km S El Frijol Colorado, 19.572, -97.383, 2,435 m, LSUMZ 36663 Cytb, 12S, GHR, IRBP.

Peromyscus attwateri

OKLAHOMA: McIntosh Co., 3.1 mi. E Dustin, 32.270, -95.975, 821 ft., TTU 55688, AF155384 Cytb only.

Peromyscus beatae

GUERRERO: Carrizal del Bravo, 17.266, -99.733, 3,625 ft., MZFC 9364 Cytb, –, GHR, IRBP.

Peromyscus leucopus

CONNECTICUT: (T-175) X99463 12S.

Peromyscus levipes

OAXACA: San Martín Caballero, Distrito de Teotitlán, 17.057, -96.734, 5,069 ft., MZFC 8726, –, –, GHR, IRBP.

Peromyscus truei truei

APPENDIX 4.2

Specimens included in the *BEAST analysis of divergence times (Drummond and Rambaut 2007). Specimens are arranged by taxon, state, collecting locality, geographic coordinates, elevation, catalogue number, and GenBank number for Cytb sequence. Specimens were obtained from the Angelo State Natural History Collection (ASNHC), Brigham Young University (BYU), the Royal Ontario Museum (ROM), and other museum collections listed in Appendix 4.1. Specimens from Appendix 4.1 used in the *BEAST analysis include CNMA 44001, CNMA 45643, MSB 48177, MSB 54457, MSB 63666, TCWC GK3076, and TTU 104423.

*Habromys ixtlani*

OAXACA: Llano de la Flores, km 132 on highway from Tuxtepec–Oaxaca, 17.09, -96.70, 5,470 ft., CNMA 29849, EF989941.

*Isthmomys pirrensis*

PANAMA: Darien Province, summit of Cerro Pirre, 7.933, -77.716, 396 ft., ROM 116308, EF989945.

*Megadontomys thomasi*

OAXACA: 11 km SW La Esperanza, 17.561, -96444, 8,147 ft., CNMA 29186, EF989948.

*Neotomodon alstoni*

Onychomys arenicola
TEXAS: Presidio Co., Hip O Ranch, 5 mi. W Marfa, 30.308, -104.10, 4,805 ft.,
ROM 114904, EF989954.

Onychomys leucogaster
TEXAS: Cameron Co., 11 mi. N Port Isabel, 26.2, -97.2, 50 ft., ASNHC 4348,
EF989958.

Osgoodomys banderanus
MICHOCAL: 8 km N La Mira, 18.107, -102.328, 322 ft., ASNHC 2664,
EF989956.

Peromyscus attwateri
OKLAHOMA: McIntosh Co., 3.1 mi. E Dustin, 32.270, -95.975, 821 ft., TTU
55688, AF155384.

Peromyscus crinitus
UTAH: Uintah County, Bitter Creek Canyon, 41.1, -111.9, BYU 16629, EF989973.

Peromyscus eremicus
SONORA: 22 km S (by road) Hermosillo, 28.889, -110.963, 755 ft., BYU 17952,
EF989975.

Peromyscus levipes
CHIAPAS: Cerro Tzontehuiz, 16.7, -92.6, 7,513 ft., ROM 97624, EF989981.

Peromyscus maniculatus
CANADA: Ontario: Kwataboahegan, Kwataboahegan River, 51.15, -80.50, 20 m,
ROM 98941, EF989983.
Peromyscus melanophrys

Stock animal from Peromyscus Genetic Stock Center, University of South Carolina, USC-PGSC XZ 1073, EF989989.

Peromyscus melanotis

Stock animal from Peromyscus Genetic Stock Center, University of South Carolina, USC-PGSC 25, EF989990.

Reithrodontomys sumichrasti

GUATEMALA: Huehuetenango: 10 km SW Santa Eulalia, 15.673, -91.528, 8,663 ft., ROM 98383, EF990023.
APPENDIX 5.1

Specimens examined.—Species name, collection locality, geographic coordinates, elevation, and catalogue number are listed for specimens used in this analysis of *Dipodomys phillipsii*. Specimens used to develop the morphological key are indicated by “(m)” following the catalogue number. GenBank numbers are listed for each specimen used in the molecular analyses. Mammal collections housing voucher or tissue specimens are ASNHC = Angelo State Natural History Collection, Angelo State University; CNMA = Colección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México; ENCB = Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; LSUMZ = Louisiana State University, Museum of Natural Science; M = Louisiana State University, Museum of Natural Science Mammal Tissue Collection; LVT = University of Nevada, Las Vegas; MLZ = Moore Laboratory of Zoology, Occidental College; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; MWSU = Midwestern State University; NMMNH = New Mexico Museum of Natural History; TTU = Museum of Texas Tech University; UAM-I = Universidad Autónoma Metropolitana-Iztapalapa; UATX = Universidad Autónoma de Tlaxcala; and USNM = United States National Museum of Natural History. Numbers in parentheses refer to localities mapped in Fig. 5.1.

*D. p. oaxaca*

D. p. ornatus

Aguascalientes: (4) 8.8 km N, Las Fraguas, 22.233, -102.083, 2,150 m, IPN 36320, Cytb = JN183912; Durango: (1) 5.8 km N, 2.1 km E Vicente Guerrero, 23.783, -103.962, 1,937 m, CNMA 39681, Cytb = JN183913, 12S = JN208380, GHR = JN661654, IRBP = JN661676; TTU 75585, IRBP = GU955164; (2) 2.2 km S, 2.5 km E Vicente Guerrero, 23.711, -103.957, 1930 m, CNMA 39683, Cytb = JN183914, 12S = JN208382, GHR = JN661655, IRBP = JN661677; Durango, 24.0, -104.6, USNM 94622 (m) and 94624 (m); Jalisco: (5) 4 km W Guadalupe Victoria, 21.699, -101.617, 2,185 m, CNMA 43385, Cytb = JN183915, 12S = JN208384, GHR = JN661656, IRBP = JN661678; Lagos [de Moreno], 21.4, -101.9, USNM 78952 (m); Querétaro: Tequisquiapan, 20.5, -99.9, USNM 78430 (m) and 78431 (m); San Luis Potosí: 1 km N Arenal [de Morelos], 22.42, -101.27, LSUMZ 5178 (m); Bledos, 21.8, -101.1, LSUMZ 5177 (m); About 1 mi. W Bledos, 21.84, -101.13, LSUMZ 4286 (m); (6) Las Cabras, 4.6 mi. NW Bledos, 21.800, -100.933, 1,820 m, LVT 2056, Cytb = AY926376; Zacatecas: (3) 2 mi. E San Jeronimo, 22.640, -97.231, 2,350 m, CNMA 42049, Cytb = JN183917, 12S = JN208385, GHR = JN661657, IRBP = JN661679; CNMA 42050, 12S = JN208385; CNMA 42121, Cytb = JN183918, 12S = JN208386, GHR = JN661658, IRBP = JN661680; Berriozábal, 22.5, -102.3, USNM 79506 (m); Hacienda San Juan Capistrano, 22.6, -104.1, USNM 90804 (m); [El] Plateado [de Joaquín Amaro], 21.9, -103.1, USNM 90808 (m); Valparaíso, 22.8, -103.6, USNM 91933 (m) and 91945 (m); Zacatecas, 22.8, -102.6, USNM 120167 (m).

D. p. perotensis
Puebla: (10) 4.5 km S, 9.5 km San José Alchichica, 19.766, -97.316, 2,350 m, IPN 42239, Cytb = JN183899; (11) 2 km W Guadalupe Victoria, 19.280, -97.378, 2,406 m, CNMA 43986, Cytb = JN183904, 12S = JN208381, GHR = JN661659, IRBP = JN661670; CNMA 43987–43988 (m); (12) 3.1 km SW Veladero, 20.591, -97.600, 2,340 m, LSUMZ 36253 (m), Cytb = JN183898; (16) 11 km (by road) SW Alchichica, 19.766, -97.316, 2,449 m, LSUMZ 36244 (m), Cytb = JN183900; (13) 6.7 km E Techachalco, 19.390, -97.408, 2,500 m; UAMI 17258, Cytb = JN183901; Chalchicomula (= Ciudad Serdán), 19.0, -97.4, USNM 53323–53328 (m) and 53333 (m); Tlaxcala: Huamantla, 19.3, -97.9, USNM 53635 (m) and 53637 (m); (8) 2.5 km NW El Carmen Tequexquitla, 19.350, -97.665, 2,378 m, CNMA 44321, Cytb = JN183902, 12S = JN208378, GHR = JN661660, IRBP = JN661674; (17) 6 km NE Cuapiaxtla, 19.300, -97.766, 2,425 m, UAT M0366, Cytb = JN183903, 12S = JN208379, GHR = JN661661, IRBP = JN661673; Veracruz: (9) 3 km S El Frijol Colorado, 19.572, -97.383, 2,435 m, CNMA 43972, Cytb = JN183905, IRBP = JN661675; CNMA 43974 (m); Perote, 19.6, -97.2, USNM 54281–54283 (m).

*D. p. phillipsii*

Mexico: (7) 5 km SE Nopaltepec, 19.753, -98.670, 2,400 m, UAMI 2779, Cytb = JN183911.

*D. agilis*

Baja California: 6 km S, 17 km E, Valle de la Trinidad, 31.754, -116.364, 905 m, MVZ 153957, Cytb = U65303.
D. californicus
California: Tehama County, 2.5 miles S, 0.2 miles E Paynes Creek, 40.306, -121.904, 1,960 feet, MLZ 2061, Cytb = AY926368.

D. compactus
Texas: Cameron County, 4.5 miles N, 3.6 miles E Port Isabel, 26.13, -97.16, ASNHC 4327, Cytb = AY926379.

D. deserti
Nevada: Clark County, Corn Creek Desert Wildlife Refuge, 39.55, -119.46, NMMNH 5374, Cytb = AY926381.

D. elator
Texas: Cottle County, 1.6 km N, 1.8 km E junction FM 1033 and FM 104, 34.250, -100.050, 500 m, TTU 45633, Cytb = AF172834, JN183919, 12S = JN208373, GHR = JN661664, IRBP = JN661688; Wichita County, 8.6 miles N Iowa Park; 34.056, -98.746, 1117 feet, MWSU17542, Cytb = JN661645, 12S = JN208374, GHR = JN661665, IRBP = JN661687.

D. heermanni
California: San Luis Obispo County, 15.0 miles S, 8.2 miles E Simmler, 33.134, -119.839, 2,300 feet, MLZ 1852, Cytb = AY926369.

D. merriami
Arizona: Maricopa County, 11.2 km N Gila Bend, 33.666, -111.866, 224 m, TTU 41781, Cytb = AF173502; Texas: Brewster County, Elephant Mountain Wildlife Management Area, 29.66, -103.35, 3562 feet, TTU 97980, IRBP = GU985162; Presidio County, Las Palomas Wildlife Management Area, 26.40, -97.80, 33 feet, TTU 75675, Cytb =

137
AF172837. Coahuila: 6 mi. E Parras, 24.86, -100.18, 2,118 m, M-8688, Cytb = JN661644, 12S = JN661641, GHR = JN661662, IRBP = JN661686. Durango: 3 mi. N Lazaro Cardenas, 25.39, -103.98, 1,500 m, M-8708, Cytb = JN661643, 12S = JN661640, GHR = JN661663, IRBP = JN661685.

D. microps

California: Inyo County, 6.0 miles N, 0.5 miles W Bishop, 37.450, -118.404, 4,200 feet, MLZ 1765, Cytb = AY926385.

D. nelsoni


D. ordii

New Mexico: Grant County, 2.6 miles N, 1.8 miles E Redrock, 32.724, -108.707, 1,241 m, NMMNH 4377, Cytb = AF173501. Luna County, 4.0 mi. S, 9.5 mi. W Deming, 33.760, -108.780, 6,198 feet, MVZ 150772, Cytb = JN661646, 12S = JN661636, GHR = JN661669, IRBP = JN661684; MVZ 150775, Cytb = JN661647, 12S = JN661637, GHR = JN661668, IRBP = JN661683.

D. panamintinus

California: San Bernardino County, 8.9 miles N, 1.1 miles E Red Mountain, 35.486, -117.595, 3,150 feet, MLZ 1879, Cytb = AY926384.
D. spectabilis


Heteromys irroratus

Puebla: 6 km N Tilapa, 18.648, -98.553, 1,300 m, LSUMZ 36295, *Cy tb* = GU647037.
## APPENDIX 5.2

List of primers used for the amplification of nuclear (*GHR* and *IRBP*) and mitochondrial (*Cytb* and *12S*) genes in the Phillips’ kangaroo rat (*Dipodomys phillipsii*).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVZ-05</td>
<td><em>Cytb</em></td>
<td>CGAAGCTTGATATGAAAAAACATCGTTG</td>
<td>Irwin et al. 1991</td>
</tr>
<tr>
<td>H15915</td>
<td><em>Cytb</em></td>
<td>AACTGCAGTCATCTCCGTTTACAAGAC</td>
<td>Irwin et al. 1991</td>
</tr>
<tr>
<td>MVZ-04</td>
<td><em>Cytb</em></td>
<td>GCAGCCCCCTCAGAATGATTTTGTCCTC</td>
<td>Smith and Patton 1993</td>
</tr>
<tr>
<td>MVZ-45</td>
<td><em>Cytb</em></td>
<td>ACJACHATAGCJACAGCATTCGTAGG</td>
<td>Smith and Patton 1993</td>
</tr>
<tr>
<td>MVZ-16</td>
<td><em>Cytb</em></td>
<td>AAATAGGAARTATCAYTCTGGTTTRAT</td>
<td>Smith and Patton 1993</td>
</tr>
<tr>
<td>MVZ-17</td>
<td><em>Cytb</em></td>
<td>ACCTCCTAggAgAYCCAgAHAAYT</td>
<td>Smith and Patton 1993</td>
</tr>
<tr>
<td>MVZ-14</td>
<td><em>Cytb</em></td>
<td>GGTCTTCATCTYHGGYTTACAAGAC</td>
<td>Smith and Patton 1993</td>
</tr>
<tr>
<td>12S L82</td>
<td><em>12S</em></td>
<td>CATAGACACACAGAGTTTGGTCC</td>
<td>Nedbal et al. 1994</td>
</tr>
<tr>
<td>12S H900</td>
<td><em>12S</em></td>
<td>TGACTGCAGAGGACGGTTGGTGTGT</td>
<td>Nedbal et al. 1994</td>
</tr>
<tr>
<td>GHR1f</td>
<td><em>GHR</em></td>
<td>GGRAARTTRGAGGAGGRGAACAC</td>
<td>Jansa et al. 2009</td>
</tr>
<tr>
<td>GHRend1F</td>
<td><em>GHR</em></td>
<td>GTTTTTGTTCAGTTGGTCTCTGCT</td>
<td>Jansa et al. 2009</td>
</tr>
<tr>
<td>IRBP-A</td>
<td><em>IRBP-A</em></td>
<td>ATGGCCAACGTCCTTGGATAAC</td>
<td>Stanhope et al. 1992</td>
</tr>
<tr>
<td>IRBP-B</td>
<td>IRBP-B CGCAGGTCCATGATGAGGTGCTCC</td>
<td>Stanhope et al.</td>
<td></td>
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<tr>
<td>--------</td>
<td>---------------------------------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1992</td>
<td></td>
</tr>
</tbody>
</table>
VITA

Jesús Abraham Fernández Fernández was born in Mexico City to Miguel Angel and Heriberta Fernández. He grew up in the city of Santa Ana Chiautempan, Tlaxcala, México, where he graduated from Escuela Secundaria Técnica No. 4. He received his bachelor’s degree in biology from Universidad Autónoma de Tlaxcala in 2000 working on bird diversity. In 2002, he began graduate school at the National University of Mexico, where he obtained the title of Master in Science under the tutelage of Dr. Fernando Cervantes. His thesis topic was phylogenetics and biogeography of kangaroo rats. In August of 2006, Jesús Abraham Fernández Fernández entered the Graduate School of Louisiana State University. Under the supervision of Dr. Mark S. Hafner, Jesús will graduate with the degree of Doctor of Philosophy in December, 2011.