Evolutionary and ecological dynamics of rapid radiation of pocket gophers (Thomomy's) in Mexico

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EVOLUTIONARY AND ECOLOGICAL DYNAMICS OF A RAPID RADIATION OF POCKET GOPHERS (THOMOMYS) IN MEXICO

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Biological Sciences

by

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B.S., North Carolina State University, 2000
M.S., New Mexico State University, 2006
May 2013
This work is dedicated to my parents, Karen and Jeffery Mathis, my stepmother Diane Mathis, and my siblings Lindsey Mathis Jones, Merida L. Mathis, and Simon P. Gaskins. Their emotional support in my educational journey helped guide me to the place I am today. This work is dedicated to the beloved memories of my grandparents, Dr. James L. Mathis and Ann K. Mathis, my aunt Leslie Mathis Gray, and my uncle Mark A. Mathis. Finally, this work is also dedicated to the memory of my dearly beloved four-legged canine friend, Panda.
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TABLE OF CONTENTS

ACKNOWLEDGMENTS………………………………………………………………………… iii

ABSTRACT…………………………………………………………………………………… vii

CHAPTER

1. INTRODUCTION………………………………………………………………………………… 1

2. RESURRECTION AND REDESCRIPTION OF THE POCKET GOPHER
   THOMOMYS SHELDONI FROM THE SIERRA MADRE OCCIDENTAL OF MEXICO
   2.1 Introduction…………………………………………………………………………………………… 7
   2.2 Materials and Methods……………………………………………………………………… 10
   2.3 Results………………………………………………………………………………………… 16
   2.4 Discussion……………………………………………………………………………………..… 26

3. THOMOMYS NAYARENSIS, A NEW SPECIES OF POCKET GOPHER FROM THE
   SIERRA DEL NAYAR, NAYARIT, MEXICO
   3.1 Introduction………………………………………………………………………………………… 34
   3.2 Materials and Methods……………………………………………………………………… 35
   3.3 Results………………………………………………………………………………………… 40
   3.4 Discussion………………………………………………………………………………………… 44

4. EVOLUTION AND PHYLOGEOGRAPHY OF THE THOMOMYS UMBRINUS
   SPECIES COMPLEX (RODENTIA: GEOMYIDAE)
   4.1 Introduction………………………………………………………………………………………… 50
   4.2 Materials and Methods……………………………………………………………………… 53
   4.3 Results………………………………………………………………………………………… 59
   4.4 Discussion………………………………………………………………………………………… 67

5. THE ROLES OF NICHE CONSERVATISM AND COMPETITION IN A RAPID
   RADIATION OF FOSSORIAL MAMMALS
   5.1 Introduction………………………………………………………………………………………… 79
   5.2 Materials and Methods……………………………………………………………………… 83
   5.3 Results………………………………………………………………………………………… 88
   5.4 Discussion………………………………………………………………………………………… 94

6. CONCLUSIONS…………………………………………………………………………………… 99

LITERATURE CITED……………………………………………………………………………… 103

APPENDIX

2.1. LIST OF SPECIMENS EXAMINED IN CHAPTER 2…………………………………… 119

2.2. LIST OF PRIMERS AND THEIR ANNEALING TEMPERATURES………………. 125
ABSTRACT

Understanding biodiversity is one of the driving foundations of evolutionary biology and researchers use a myriad of tools to uncover and understand the processes contributing to it. The evolutionary and ecological dynamics in a group of smooth-toothed pocket gophers, the *Thomomys umbrinus* species complex, is studied for this dissertation. This complex is distributed from south-central Arizona and southwestern New Mexico south to Veracruz, Mexico. The genetic complexity of *T. umbrinus* was initially discovered via allozymes and karyotypes, resulting in five genetic clades: three with one diploid number of chromosomes (2n = 76; two clades distributed in the Sierra Madre Occidental and 1 along the Pacific Coast) and two with a different diploid number (2n = 78; one in the Northern Desert and one in the Central Plateau).

Analyses of DNA sequences from 8 genes and genotype assignment tests for 21 allozyme loci establish the Sierra Madre clade within what was formerly *T. umbrinus* as a genetically isolated taxon. Accordingly, *Thomomys sheldoni* Bailey, 1915 is resurrected to recognize this divergent clade of pocket gophers with a diploid number of 2n = 76. A synonymy is provided for two subspecies within *T. sheldoni* based on a concordant genetic and morphological break.

Multi-locus genetic analyses reveal a previously undescribed species of pocket gopher (2n = 76) apparently restricted to the Sierra del Nayar of northeastern Nayarit. Molecular, chromosomal, and cranial morphometric data distinguish this new species from other members of the *T. umbrinus* species complex. This new taxon, *T. nayarensis*, is described and a key to distinguishing the 3 species of *Thomomys* in northeastern Nayarit is provided.

Subspecies relationships within *T. umbrinus* (2n = 78) are reevaluated using phylogenetic analyses, species tree analyses, allozymes, and morphology. Phylogenetic analyses confirm three
genetic clades (Northern Desert, Central Plateau, and the Trans-Mexico Volcanic Belt [TMVB]). Reanalysis of published allozyme data shows no evidence of nuclear discordance among the three clades. Species tree analyses reveal four divergent lineages (two within the TMVB clade), which are recognized herein at the subspecies level.

Species distribution models were used to assess biotic and climatic factors that may influence how members of the *T. umbrinus* complex are distributed. *T. sheldoni* and *T. atrovarius* had well-predicted niches and climatic variables that differentiated them from the *T. umbrinus* clades. Niche equivalency tests were rejected and evidence of niche conservatism was found between some, but not all, members of the species complex, indicating a complex history of niche evolution, competition, and genetic differentiation in the *T. umbrinus* species complex.
CHAPTER 1
INTRODUCTION

Understanding biodiversity is one of the driving foundations of evolutionary biology. How, when, and why species evolve has long fascinated those in the sciences. Speciation research began in earnest after Darwin’s (1859) seminal publication, then saw a resurgence of interest with the advent of the Modern Synthesis (Huxley 1942). We are now in the middle of a third phase of speciation research, one in which modern genetic techniques and analytical methods provide better answers to deeper questions, while ultimately raising myriad new questions about the speciation processes (Coyne and Orr 2004).

Adaptive radiations, wherein new clades evolve rapidly to fill novel niche space or function, are well-known and well-studied aspects of the speciation process (e.g., Baldwin and Sanderson 1998; Losos and Miles 2002; Rainey and Travisano 1998; Schluter 2000; Seehausen 2004). However, radiations can also be non-adaptive, in which case diversification within a lineage occurs without niche differentiation, often resulting in allopatric species that occupy similar niches (Gittenberger 1991, 2004; Kozak et al. 2006; Rundell and Price 2009).

There are many tools researchers can use to identify and understand non-adaptive radiations, ranging from morphological to ecological to molecular. The most commonly used methods today are molecular phylogenetics and molecular phylogeography, wherein genetic processes are explored in an historical or geographical context (Avise 2000; Felsenstein 2004). However, as powerful as molecular methods are, radiations are often difficult to resolve with molecular data because synapomorphic changes are rare or absent on short, internal branches of the phylogeny, resulting in lack of resolution at key nodes (Steppan et al. 2004). Incomplete lineage sorting is also a potential problem in studies of rapid radiations, especially recent radiations, because gene trees may not reflect the true species tree because of shared ancestral
alleles (Whitfield and Lockhart 2007). Recent molecular advances, including high-throughput sequencing and coalescent-based species tree analyses, may alleviate some of the difficulty in using molecular methods to understand speciation and rapid radiations (Belfiore et al. 2008; Noor and Feder 2006; Whittall et al. 2006).

Species radiations can also be assessed via ecological and biogeographical methods. Whereas the species produced by an adaptive radiation generally occupy unique niches, often sympatrically (Schluter 1996), species resulting from a non-adaptive radiation usually occupy similar niches in allopatry. This means that studies of species distributions and niche characteristics may lend key insight into how and why they radiated. New and useful tools, such as ecological niche modeling, are providing unprecedented views into the history of phyletic radiations, even allowing reconstruction of past climates to investigate the tempo and mode of radiations (Austin 2007). Ecological niche models also allow tests of niche overlap and conservatism, which may be key factors in distinguishing between adaptive and non-adaptive radiations.

For this dissertation, I set out to explore a potentially non-adaptive rapid radiation in a genus of fossorial rodents. Pocket gophers (family Geomyidae) are distinctive among North American mammals because they live almost their entire lives underground and show high, almost unrivaled levels of genetic and chromosomal variation. This degree of variation in Geomyidae results from several interacting life history characteristics, including low dispersal capabilities and small, patchily distributed populations (Patton and Smith 1989). Inter-population genetic differentiation in pocket gophers can exceed levels recorded between well-established species of mammals (Patton and Yang 1977; Zimmerman and Gayden 1981), meaning that degree of genetic divergence and taxonomic status of gopher populations are largely decoupled.
To further complicate matters, pocket gophers are susceptible to genetic reticulation; two species in the genus *Thomomys* (*T. bottae* and *T. townsendii*) exemplify this problem. In this case, rampant paraphyletic and polyphyletic relationships have been uncovered for both mitochondrial and nuclear data (Patton and Smith 1993, 1994). In such instances, it becomes difficult to decide whether processes, such as gene flow, or patterns, such as monophyly, should guide taxonomic decisions (Patton and Smith 1994).

The Geomyidae family is thought to have undergone a rapid radiation during their evolution (Belfiore et al. 2008; Spradling et al. 2004). They first appear in the fossil record in the late Miocene and appear to have experienced a rapid radiation in the Pliocene during the Blancan, where the number of genera more than doubled from the previous North American Land Age (Korth 1994). The molecular work of Spradling et al. (2004) reinforced the idea that geomyids evolved rapidly, possibly in response to the increased grasses available followed by increased habitat patchiness resulting from Plio-Pleistocene climate changes (Webb and Opdyke 1995).

This study focuses on the nominal species that comprise the *Thomomys umbrinus* species complex, distributed primarily in Mexico with populations also found in southeastern Arizona and southwestern New Mexico. Members of the *Thomomys umbrinus* species complex belong to the subgenus *Megascapheus* along with *T. bottae*, *T. bulbivorus*, and *T. townsendii* (Fig. 1.1). The *T. umbrinus* complex has been the subject of relatively little research compared to its widespread northern congener, *T. bottae*. Previous research based on chromosomal and allozyme data identified 5 genetic clades within what was then considered a single species, *T. umbrinus* (Hafner et al. 1987; Patton and Feder 1978). Little research was focused on this clade until
Fig. 1.1.—General distributions of the five *Thomomys* species in subgenus *Megascapheus* in western United States and Mexico. *Thomomys atrovarius* is the most recent addition to the subgenus (Hafner et al. 2011).

publications by Álvarez-Castañeda (2010) and Hafner et al. (2011) resurrected the western-most clade of *T. umbrinus* to species status as *T. atrovarius*.

In Chapter 2, I focus on 2 genetic clades within the *T. umbrinus* species complex found in the Sierra Madre Occidental of Mexico, first identified by Patton and Feder (1978) and later formally designated as the Northern and Southern Sierra Madre clades by Hafner et al. (1987). Chromosomal and allozymic analyses identified these clades as possibly genetically isolated
from other *T. umbrinus*, specifically the clade found immediately north of the Northern Sierra Madre clade, called the Northern Desert clade. I use multi-locus genetics, allozymes, and morphology to characterize this genetically divergent clade and resolve previous questions about its species status.

In Chapter 3, I recognize and formally describe a new species of *Thomomys*, *T. nayarensis*, previously unrecognized by science. This species occurs only in a remote region of northeastern Nayarit and is currently known from only 2 populations. The addition of *T. nayarensis* to the *T. umbrinus* species complex is further evidence of what could be a rapid phyletic radiation within this group.

In Chapter 4, I examine relationships within *T. umbrinus*, with the focus on what criteria should be used to define subspecies. For many taxa, subspecies may be designated based on morphology, pelage characteristics, distribution, genetics, or some combination of these. In this chapter I use a combination of traditional phylogenetic analyses, species tree analyses, and morphology to determine the status of the 18 subspecies currently recognized within *T. umbrinus*. Unlike many previous studies focused at the subspecies level, I provide logical and explicit criteria for subspecies recognition. I also provide estimates of divergence dates within the complex based on fossil calibrations that provide evidence of the relatively fast evolution of this complex and use those dates to generate a phylogeographic hypothesis of the clade’s origin and subsequent range expansion Mexico.

Finally, in Chapter 5, I use ecological niche modeling to analyze the ecological conditions that might predict where members of the *T. umbrinus* species complex may occur and how niche conservatism may have evolved in each species. Incorporating museum collection records with recent localities generated in my own fieldwork, I explore whether the distribution
of a fossorial mammal largely buffered from the outside environment can be predicted based on abiotic factors alone. I also explore the role potential niche conservation between closely related species may have played in diversification, and I address the potential role of competition in structuring current distributions.

Together, these chapters attempt to shed light on a divergent group of mammals that have contributed to the already incredible biodiversity of Mexico. A clearer understanding of the molecular phylogeny, systematics, and ecological history of these species contributes to our overall knowledge of speciation processes and how life history attributes can shape diversity.
CHAPTER 2

RESURRECTION AND REDESCRIPTION OF THE POCKET GOPHER THOMOMYS SHELDONI FROM THE SIERRA MADRE OCCIDENTAL OF MEXICO

2.1 INTRODUCTION

The southern pocket gopher, Thomomys umbrinus Richardson, 1829, as recognized by Patton (2005), is a primarily Mexican rodent that is poorly studied relative to its northern congener, T. bottae. T. umbrinus is distributed from south-central Arizona and southwestern New Mexico southward into Veracruz, Mexico (Fig. 2.1). The published records of T. umbrinus are fraught with taxonomic controversy. Because of documented hybridization between T. umbrinus and T. bottae in southern Arizona (Hoffmeister 1969; Patton and Dingman 1968), Hall (1981) considered these taxa conspecific and listed >200 subspecies of T. umbrinus. Taxonomic references now recognize T. umbrinus and T. bottae as separate species, with T. umbrinus containing 25 valid subspecies (Patton 2005). At least 8 subspecies of T. umbrinus originally were described as species, of which one was recently returned to species status as T. atrovarius (Álvarez-Castañeda 2010; Hafner et al. 2011).

As the T. bottae - T. umbrinus controversy illustrates, pocket gopher taxonomy is complicated by the fact that well-recognized species often hybridize when in contact (Hafner et al. 1983; Patton 1973; Patton et al. 1979; Patton et al. 1984; Thaeler 1968, 1974). Patton and Smith (1989) differentiated between species and races of pocket gophers based on levels of hybridization. They recognized taxa as species if hybridization was limited to F1 hybrids, which indicated absence of genetic introgression. Patton (1993) recognized that pocket gopher taxa could experience limited genetic introgression on a local scale yet still be on separate evolutionary trajectories at a broader geographical scale because of the decreasing effect of gene

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1 Reprinted by permission of Journal of Mammalogy (Appendix 2.5)
Fig. 2.1.—Distribution of *Thomomys umbrinus* clades in Mexico and southwestern United States (outlined in black) and adjoining distribution of *T. bottae* (striped) showing the location of samples used in the genetic analyses (Appendix 2.1). Black dots show capture localities of specimens used only in the analysis of the cytochrome *b* gene. White dots with numbers show the localities of specimens used in the full, multi-locus analysis. Triangles are localities of ancient DNA samples. The 2 black stars (localities 35 and 36) indicate samples of *T. bottae* used as outgroups. Clades were originally defined by Hafner et al. (1987) based on allozyme and chromosomal data. Major changes include discovery of 2 genetically defined clades within the old Central Plateau clade, assignment of localities 8–10 to the Central Plateau clade rather than the Northern Desert clade, merger of the Northern and Southern Sierra Madre clades into a single clade now recognized as *T. sheldoni*, and discovery of a genetically divergent 2n = 76 clade of *Thomomys* in Sierra del Nayar clade. The distribution of *T. atrovarius* is modified from Fig. 1 in Hafner et al. (2011). The 2 question marks flanked by dashed lines in the Sierra Madre and Trans-Mexico Volcanic Belt clades indicate regions with no museum records of *Thomomys* pocket gophers.
flow with increasing distances between populations. To make matters more complicated, divergent lineages of pocket gophers have been shown to be susceptible to genetic reticulation, where paraphyletic and polyphyletic relationships may be uncovered for both mitochondrial and nuclear data. These conflicting results may lead to potentially fully resolved yet incorrect species trees (Patton and Smith 1993, 1994).

Patton and Feder (1978) used chromosomes and allozymes to identify 3 distinct groups within *T. umbrinus* that might represent separate species: 1 with a diploid chromosome number (2n) of 78 found mostly on the Mexican plateau but extending into southwestern New Mexico and southeastern Arizona, and 2 higher-elevation groups with 2n = 76 that were potentially separated by the Barranca del Cobre (“Copper Canyon”) in the Sierra Madre Occidental of Mexico.

Patton and Feder’s (1978) findings were corroborated by Hafner et al. (1987) who, with broader sampling, used allozymes and karyology to delimit 5 geographic groups within *T. umbrinus*. Three groups had 2n=76: the previously identified Northern and Southern Sierra Madre groups and a newly discovered Coastal Sinaloa group possibly basal to the entire *T. umbrinus* complex. The 2n=78 group on the Mexican plateau was divided into Central Plateau and Northern Desert groups. These 5 groups were upheld in a genus-wide phylogenetic analysis of *Thomomys* by Smith (1998). Álvarez-Castañeda (2010) recommended that the Mexican-Pacific clade (equivalent to the Coastal Sinaloa group of Hafner et al. 1987) be elevated to species status, and Hafner et al. (2011) formally resurrected the name *T. atrovarius* J. A. Allen 1898 for this taxon. Álvarez-Castañeda (2010) further recommended elevation of a “Mexican mountain group” (corresponding to the combined Northern and Southern Sierra Madre groups)
of *T. umbrinus* to full species status as “*T. chihuahu[a]e*” [sic], but did not sample critical type localities in his analysis, which rendered his recommendation problematic.

Further investigation of the species status of the Sierra Madre clades of *T. umbrinus* is clearly warranted given the presence of fixed chromosomal and allelic differences between the Northern Sierra Madre (2n = 76) and Northern Desert (2n = 78) groups where they come into close proximity in northwestern Chihuahua (Hafner et al. 1987, Fig. 2.1). Allozymic differences between the Central Plateau and Northern Desert groups (both 2n = 78) signal the need for additional investigation of the species status of these groups as well.

In this study, I investigate the evolutionary relationships among 4 clades of *T. umbrinus* (Northern Sierra Madre, Southern Sierra Madre, Northern Desert, and Central Plateau—Hafner et al. 1987) by examination of new DNA sequence data, reanalysis of published allozyme data, and study of cranial morphometrics. I use existing tissue and skeletal material of *T. umbrinus* available from museum collections supplemented by new samples obtained through extensive fieldwork in Mexico to improve my understanding of the geographic distribution of the genetically defined clades within the *T. umbrinus* complex.

### 2.2 MATERIALS AND METHODS

**Sampling.**—124 specimens of *Thomomys* (including 90 *T. umbrinus*, 21 *T. atrovarius*, and 13 *T. bottae*) were collected between 2006 and 2012 using standard trapping methods approved by the American Society of Mammalogists (Sikes et al. 2011). Selected individuals from each locality were karyotyped in the field using the postmortem technique of Hafner and Sandquist (1989) to verify diploid numbers. Vouchers were prepared as skin-plus-skeleton specimens (Hafner et al. 1984) and deposited in the Louisiana State University Museum of Natural Science (LSUMZ) or the Colección Nacional de Mamíferos, Instituto de Biología,
Universidad Nacional Autónoma de México (CNMA). Frozen tissues from an additional 90 Thomomys individuals were obtained from museum tissue collections. Collection localities are listed in Appendix 2.1 and mapped in Fig. 2.1.

**DNA sequencing.**—In the initial phase of the analysis, the mitochondrial cytochrome b gene (Cytb) was sequenced for at least 1 individual from each locality. Based on those results, DNA sequences for 7 additional genes were obtained for 31 T. umbrinus individuals chosen to represent the overall geographic distribution of each clade (Fig. 2.1). Also included in the final dataset were 3 specimens of T. atrovarius and 2 specimens of T. bottae (representing, along with T. umbrinus, the subgenus Megascapheus). Outgroups included 1 specimen each of T. talpoides and T. mazama (representing the subgenus Thomomys) and 1 specimen of Orthogeomys hispidus (Appendix 2.1).

DNA sequences were obtained from 3 mitochondrial genes: Cytb (1,140 base pairs [bp]), 12S rRNA (12S; 868 bp), and cytochrome oxidase I (COI; 1,545 bp). In addition, 5 nuclear genes were sequenced, including the 5’ end of exon 1 of the single-copy interphotoreceptor retinoid binding protein (IRBP; 1,272 bp), the growth hormone receptor gene (GHR; 832 bp), recombination activating protein I (RAG1; 1,293 bp), the mast cell growth factor protein (MGF; 729 bp), and 1 anonymous locus (TBO47 from Belfiore et al. 2008; 601 bp).

DNA was extracted from approximately 25 mg of liver or kidney tissue using the DNeasy extraction kit (Qiagen, Valencia, California), following the protocol for animal tissues. DNA was amplified using the following polymerase chain reaction (PCR) conditions in a 25 µl reaction volume: 1–2 µl (50 ng) template DNA, 0.5 µl of 10 mM dNTPs (2.5 mM each of dATP, dCTP, dGTP, dTTP), 0.5 µl of primer (primers are listed in Appendix 2.2), 2.5 µl MgCl₂ (25 mM), 1 µl 1X BSA, 2.5 µl 10X buffer, 0.1 µl Taq (Amplitaq Gold DNA polymerase, Applied Biosystems,
Foster City, California), and sterile dH₂O. The thermal profile consisted of 95°C for 2–10 min, followed by 30–35 cycles of the following: denaturation at 95°C for 15–90 s, annealing at a primer-specific temperature (Appendix 2.2) for 20–120 s, 1–2 min extension at 72°C, and final primer extension at 72°C for 5–10 min. PCR products were visualized on 1% sodium borate agarose gels stained with ethidium bromide or Syber Green (Zipper et al. 2004). Positive amplicons were then purified with a 20% polyethylene glycol clean-up solution or an exonuclease I and shrimp alkaline phosphatase solution (ExoSAP-IT; Affymetrix Inc., Santa Clara, California).

Both DNA strands were sequenced from clean reaction products using 1.5–2.1 µl of 5X sequencing buffer (Applied Biosystems), 1 µl of 10 mM primer, 1–1.5 µl template, 0.35–0.5 µl Big Dye Terminator cycle-sequencing kit 3.1 (Applied Biosystems), and 1.5–2.1 µl of sterile dH₂O. Cycle sequencing conditions consisted of 95°C for 5 min, followed by 40 cycles of the following: denaturation at 95°C for 30 s, annealing at 50°C for 10 s, and annealing at 60°C for 4 min. Cycle sequencing product was cleaned using Sephadex G-50 (GE Healthcare, Piscataway, New Jersey) in 400 µl DTR 96-well plates (Phenix Research Products, Candler, North Carolina). Amplicons were separated and visualized on an Applied Biosystems 3100 Genetic Analyzer housed in the LSU Museum of Natural Science. Sequences were assembled and edited using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, Michigan) and Geneious 5.2 (Drummond et al. 2011). Alignments were made using the MUSCLE algorithm in Geneious and checked by eye.

Pocket gophers from 6 localities (including the type locality of *T. umbrinus sheldoni* in the Sierra Madre Occidental) were included for ancient DNA analysis. Skin clips were obtained from museum study skins collected between 1955 and 1977 and amplified for a fragment of *Cytb*. Amplification and sequencing protocols and primer information for the ancient DNA can
be found in Hafner et al. (2011). All DNA sequences are deposited in GenBank (Appendix 2.3) with the exception of 4 ancient DNA sequences that did not meet the minimum length requirement (Appendix 2.4).

Phylogenetic analyses.—Bayesian Inference (BI) analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and maximum likelihood (ML) analyses were implemented in RaxML 7.3.0 (Stamatakis 2006) via the CIPRES Gateway (Miller et al. 2010). I evaluated the most appropriate evolutionary models for each gene in MrModelTest 2.4 (Nylander 2004), which provides models appropriate for both BI and ML analyses. I selected the best model using the Akaike Information Criterion (Table 2.1).

In both sets of phylogenetic analyses (BI and ML), nucleotide sequences were concatenated and then partitioned by gene using each gene’s appropriate evolutionary model (Table 2.1). For the BI analysis, model parameters were treated as unknown variables with uniform priors. Two independent runs were initiated with random starting trees, an initial melting point of 0.25, and run for at least $9 \times 10^6$ generations with 4 incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck and Ronquist 2001) and sampled every 100 generations. Convergence and stationarity were assessed using Tracer v1.5 (Rambaut and Drummond 2007). Trees generated before stationarity of log-likelihood scores was reached were discarded. Clade support was assessed using Bayesian posterior probabilities. ML gene-partitioned analyses were run for 1,000 bootstraps, using the GTRCAT model for the bootstrapping phase in RaxML and GTRGAMMA model for the tree inference phase.

To supplement the BI and ML analyses, a species tree analysis was run in *BEAST 1.7.2* (Heled and Drummond 2010). This program co-estimates multiple gene trees within a shared species tree in a coalescent framework using a Bayesian MCMC algorithm. The same taxa and
sequences from the BI and ML analyses were used in this analysis with the exception of the
MGF gene, which lacked a representative for the outgroup Orthogeomys hispidus. Individuals
were assigned to genetic clades according to the BI and ML phylogenetic analyses. The same
evolutionary models used in the BI and ML analyses were used in the species tree analysis
(Table 2.1). Uncorrelated lognormal relaxed clock models were used and species trees were
estimated using the Yule process tree prior with a randomly generated starting tree. The analysis
was run for $10^7$ generations with sampling every 5,000 generations. Two independent runs of this
analysis were performed to assess and confirm convergence on the same species tree. Both runs
were combined using LogCombiner and convergence of the MCMC was assessed using Tracer
v1.5, where high effective sample sizes (ESS > 500) for all parameters were confirmed. After
discarding a 10% burn-in, a maximum clade credibility tree was generated in TreeAnnotator
(BEAST 1.7.2 package—Drummond and Rambaut 2007).

Genetic divergence.—Genetic divergence values and phylogenetically informative sites
were analyzed in MEGA 5 (Tamura et al. 2007). A Mantel test, implemented in Genalex 6
(Peakall and Smouse 2006) was conducted on the 2n = 78 T. umbrinus complex to test for
isolation by distance. The test was run for 999 random permutations and used matrices of
geographic distances and Kimura 2-parameter pairwise sequence divergence of Cytb to address
the hypothesis that there is a significant correlation between increasing genetic and geographic
distance.

Genotype assignment test.—Allozyme data originally detailed in Hafner et al. (1987)
were used to further investigate the relationships between the 2n = 78 Northern Desert clade and
the 2n = 76 Northern and Southern Sierra Madre clades. Genotype assignment tests for 22
polymorphic loci were performed in Arlequin 3.1 (Excoffier et al. 2005). These tests compute the log-likelihood of the genotypes of the individuals in each clade under the assumption that they were taken from the same population and have equal allele frequencies (Paetkau et al. 1997; Waser and Strobeck 1998). The output from this test can allow us to infer whether the individual genotypes belong more to one population than to another.

Morphometric analyses.—Because of extreme sexual dimorphism in pocket gophers (Hafner et al. 2004; Patton and Smith 1990; Smith and Patton 1988), only adult female Thomomys were used in the morphometric analyses. Specimens were judged to be adult based on fusion of the exoccipital-supraoccipital and basioccipital-basispheniod sutures (Daly and Patton 1986). Twelve cranial characters were measured to the nearest 0.1 mm using hand-held digital calipers. The characters measured were: cranial width (CW), diastema length (DIA), width of interorbital constriction (IOC), mastoid breadth (MB), length of maxillary tooth row (MTR), nasal length (NL), occipital-nasal length (ONL), occipital-incisor length (OIL), rostral width (RW), zygomatic breadth (ZB), breadth of mandible (BM), and mandible length (ML). These characters have proven to be informative in previous morphometric studies of pocket gophers.
Morphological variation in Sierra Madre *T. umbrinus* was analyzed by dividing the group into northern and southern subgroups based on the molecular data. These subgroups were then compared independently to geographically proximate populations of pocket gophers belonging to other groups of *T. umbrinus* or *T. atrovarius*. The northern and southern subgroups also were compared to each other to examine within-group geographical variation.

Statistical analyses of the morphometric data were conducted using SPSS 19 (IBM, Armonk, New York). Data were assessed for normality and examined for extreme outliers, which were removed from further analyses. Data were transformed ($\bar{X} = 0$, $SD = 1$) and a multivariate analysis of variance (MANOVA) was used to test the null hypothesis of no significant difference between *a priori* groups. A post hoc analysis of the MANOVA was assessed with Tukey’s HSD. Direct discriminant function analysis (DFA) was performed to generate discriminant functions to predict group membership and evaluate if individuals could be properly assigned to their *a priori* groups.

### 2.3 RESULTS

**Phylogenetic analyses.**—The 3 mitochondrial genes (*Cytb, 12S, and COI*) were explored both separately and also as a concatenated dataset because they are linked and share the same evolutionary model (Table 2.1). Separately, they produced topologically similar trees (not shown) once weakly supported nodes were collapsed (BI posterior probability $[pp] < 0.95$, bootstrap support $[bs] < 80\%$). The tree generated from the concatenated sequences (not shown) showed only moderate support for basal nodes, but showed strong support ($pp \geq 0.99$, $bs \geq 90\%$) for monophyly of the genetic clades originally defined by Hafner et al. (1987). Phylogenetic analyses conducted separately on each of the 5 nuclear genes resulted in largely unresolved trees
(trees not shown); however, \textit{GHR} and \textit{RAG1} (BI and ML analyses) and \textit{MGF} (ML only) showed support for monophyly of the Sierra Madre clade. Parsimony-informative sites ranged from 1% to 37% of each gene (Table 2.1).

Pairwise partition-homogeneity tests conducted in PAUP* (Swofford 2003) revealed that trees generated from the \textit{IRBP} sequences had a significantly different topology from trees generated from the other genes ($p \leq 0.04$). However, gene-partitioned ML and BI trees generated from the concatenated sequences showed identical topologies with or without the \textit{IRBP} sequences, so \textit{IRBP} sequences were included in all subsequent analyses resulting in a total of 8,280 bp (mitochondrial + nuclear) analyzed.

Gene-partitioned ML and BI trees generated from the concatenated sequences (Fig. 2.2) show relatively strong support for the $2n = 78$ clades previously reported by Hafner et al. (1987), with some notable differences. Two samples from north-central Chihuahua (localities 8 and 10 in Fig. 2.1) were originally classified by Hafner et al. (1987) as Northern Desert based on allozymes, but my multi-locus DNA sequence analyses indicate they belong to the redefined Central Plateau clade. As a result, the Northern Desert clade of Hafner et al. (1987) appears to be restricted to a small number of localities in New Mexico, Arizona, Sonora, and northwestern Chihuahua. The Central Plateau clade, as redefined herein, is distributed from central Chihuahua through central Durango, and there is moderate support for a sister relationship with the Northern Desert clade ($pp = 0.95$, $bs = 89$; Fig. 2.2). Increased sampling revealed a basal clade of \textit{T. umbrinus} with $2n = 78$, extending southward from southern Durango into the Trans-Mexico Volcanic Belt (TMVB) of central Mexico (Fig. 2.1). Within this clade, the northernmost locality sampled (locality 20; Fig. 2.2) appears to be basal to and genetically distinct from the other members of the TMVB.
Pocket gophers from the Sierra Madre Occidental form a well-defined monophyletic group (Figs. 2.1 and 2.2) that is part of an unresolved polytomy. Individuals of the Sierra Madre clade shared no haplotypes with individuals of any other group at any of the genes examined except TBO47, which had only 6 haplotypes for the entire dataset and was uninformative from a phylogenetic perspective.

My DNA sequence analyses revealed a genetic subdivision between northern and southern populations of the Sierra Madre Occidental, but the split does not coincide with the Barranca del Cobre, as hypothesized by Hafner et al. (1987). Instead, individuals collected on either side of this precipitous canyon (localities 12 and 13 in Fig. 2.1) belong to the same monophyletic group. Individuals from locality 15 (Figs. 2.1 and 2.2) were either weakly grouped with other northern Sierra Madre populations via ML (bs = 48) or were basal to the Sierra Madre clade via BI (pp = 0.6).

Newly collected individuals from the Sierra del Nayar of Nayarit (localities 25 and 26 in Fig. 2.1) formed a surprisingly divergent monophyletic group distinct from other 2n = 76 clades (Fig. 2.2). Despite being collected only 13 km from individuals of the Sierra Madre clade, individuals of the Sierra del Nayar clade share no haplotypes with Sierra Madre individuals at any of the genes sampled.

Results of the species tree analysis in *BEAST generally corroborated those of the BI and ML analyses, but with weaker support at almost all nodes. Only the 2n = 78 T. umbrinus clade had strong (bs > 0.90) support for monophyly.

Genetic divergence.—Mean Cytb divergence values calculated using the Kimura 2-parameter correction (Kimura 1980) show within-clade divergences ranging from 0.6% within the geographically restricted Sierra del Nayar clade to 10.5% within the widespread TMVB clade.
Fig. 2.2.—Genetic relationships among 5 clades in the *Thomomys umbrinus* complex, *T. atrovarius*, and *T. bottae* based on Bayesian and maximum likelihood analyses (8 genes; 8,280 bp) in which the sequence data were concatenated and partitioned by gene. Only the Bayesian tree topology is shown. Black circles indicate highly supported nodes, and nodes with weak support (posterior probability < 0.95 or bootstrap support < 80%) are collapsed by removal of the unsupported inter-nodal branches so as to retain correct lengths for all terminal and subterminal branches. Numbers at the tips of branches refer to localities mapped in Fig. 2.1 and listed in Appendix 2.1. The hypothesized evolution of the 2n = 78 diploid number from the presumed primitive 2n = 76 diploid number is indicated on the tree. Average percent sequence divergence (Kimura 2-parameter model) for the cytochrome *b* gene is indicated at three key nodes. Outgroups include 1 specimen of *Orthogeomys hispidus* and 2 individuals representing the subgenus *Thomomys* (*T. mazama* and *T. talpoides*). Scale bar represents the number of substitutions per site.
Mean pairwise Cytb divergence values among the 5 major clades of *T. umbrinus* averaged 15.9%. *T. atrovarius* had an average divergence of 16.4% from the 5 *T. umbrinus* clades examined, and *T. bottae* had an average Cytb divergence of 17.6% from *T. umbrinus* and *T. atrovarius*. Average divergence between the northern and southern Sierra Madre populations was 6%. A Mantel test of individuals belonging to the 2n = 78 *T. umbrinus* complex revealed a significant pattern of increasing genetic distance with increasing geographic distance, supporting an isolation-by-distance effect ($R^2 = 0.23, P = 0.001$).

**Genotype assignment test.**—The genotype assignment tests using allozyme data from Hafner et al. (1987) revealed a sharp discordance between the Northern Desert and Sierra Madre clades, with no overlap of log-likelihood scores between them (Fig. 2.3). The absence of shared genotypes is consistent with the analysis of the multi-locus sequence data in signaling genetic isolation between the 2 clades.

**Morphometric analysis.**—The Sierra Madre clade was divided into northern and southern geographic subgroups as defined in the multi-locus genetic analysis (Fig. 2.2). Specimens from locality 15 (Figs. 2.1 and 2.2) were treated as members of the northern geographic group. Northern Sierra Madre individuals were compared to proximate individuals from the Northern Desert and Central Plateau groups. Southern Sierra Madre individuals were compared to nearby samples from the Sierra del Nayar and TMVB clades, as well as nearby individuals of *T. atrovarius*. Finally, northern and southern Sierra Madre individuals were compared to each other to investigate whether morphological differences coincided with the genetic break shown in Fig. 2.2. In the comparison of southern Sierra Madre individuals with nearby populations of other groups, 2 variables (IOC and CW) were non-normally distributed, but after removal of extreme outliers, only CW remained non-normal. In the comparison of northern Sierra Madre individuals
Table 2.2.—Average (and range) percent sequence divergence (Kimura 2-parameter model) at the cytochrome *b* gene within and between clades of *Thomomys umbrinus* and nearby populations of *T. atrovarius* and *T. bottae*.

<table>
<thead>
<tr>
<th>T. <em>umbrinus</em></th>
<th>Central Plateau</th>
<th>Northern Desert</th>
<th>Trans-Mexico Volcanic Belt</th>
<th>Sierra Madre</th>
<th>Sierra del Nayar</th>
<th>T. <em>atrovarius</em></th>
<th>T. <em>bottae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T. <em>umbrinus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Plateau</td>
<td>4.5% (0.4–6.6%)</td>
<td>13.0% (10–17%)</td>
<td>15.9% (11–21%)</td>
<td>17.5% (15–21%)</td>
<td>15.9% (14–18%)</td>
<td>18.5% (14–22%)</td>
<td>17.3%</td>
</tr>
<tr>
<td>Northern Desert</td>
<td>2.6% (0.5–4.1%)</td>
<td>13.2% (11–16%)</td>
<td>15.6% (12–19%)</td>
<td>17.0% (15–19%)</td>
<td>14.2% (12–18%)</td>
<td>16.1% (14–20%)</td>
<td></td>
</tr>
<tr>
<td>Trans-Mexico Volcanic Belt</td>
<td>10.5% (1.9–15.8%)</td>
<td>16.4% (13–21%)</td>
<td>17.8% (16–20%)</td>
<td>17.0% (12–21%)</td>
<td>18.7% (13–23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sierra Madre</td>
<td>5.9% (0–11.6%)</td>
<td>16.5% (15–18%)</td>
<td></td>
<td>15.1% (13–19%)</td>
<td>16.8% (13–22%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sierra del Nayar</td>
<td>0.6% (0–1.3%)</td>
<td>17.1% (15–20%)</td>
<td></td>
<td>19.0% (15–21%)</td>
<td></td>
<td>7.9% (14–21%)</td>
<td></td>
</tr>
<tr>
<td>T. <em>atrovarius</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. <em>bottae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.4%</td>
</tr>
</tbody>
</table>


Fig. 2.3.—Average log-likelihood scores with 95% confidence intervals from genotype assignment tests for 22 polymorphic allozyme loci for specimens from the Northern Desert (2n = 78), Northern Sierra Madre (2n = 76), and Southern Sierra Madre (2n = 76) subclades of the *T. umbrinus* complex. Populations are ordered in a north-to-south direction and sample sizes follow locality names. The assignment scores are calculated as though the Northern Desert clade were the source population.
with their neighbors, ZB was non-normal. Non-normal variables were removed from the MANOVA but were retained for the DFA because this analysis is robust to deviations from normality not caused by outliers (Tabachnick and Fidell 1996).

In the comparison of northern Sierra Madre individuals with nearby individuals belonging to other groups, a MANOVA revealed that northern Sierra Madre individuals were significantly larger than nearby individuals from the Northern Desert group for all measurements \((P < 0.05)\) and significantly larger than adjacent Central Plateau individuals for ONL, RW, and IOC.

Two significant canonical discriminant functions successfully classified 95.6% of individuals to their correct group (100% for northern Sierra Madre, 81.8% for Central Plateau, and 100% for Northern Desert). Occipital-nasal length showed strong positive loading and occipital-incisor length showed strong negative loading on DF 1 in the analysis (Table 2.3). A visual inspection of DF 1 and DF 2 (Fig. 2.4a) reveals reasonably good separation of the 3 groups, with Central Plateau specimens separated from the other groups mostly along DF 1 and Northern Desert individuals separated from the other groups primarily by DF 2.

In the comparison of southern Sierra Madre individuals with nearby individuals belonging to other groups, Tukey’s HSD post hoc tests on the significant MANOVA revealed individuals from the southern end of the Sierra Madre clade to be significantly \((P < 0.05)\) larger than TMVB individuals for ONL, NL, and RW. The Sierra Madre group was significantly larger than both the TMVB group and \(T.\ atrovarius\) for IOC and ZB. Individuals of the southern Sierra Madre group were always significantly larger than individuals from the Sierra del Nayar \((P < 0.05)\) except for the variable IOC. Given the small sample size of Sierra del Nayar individuals \((n = 9)\), this apparent size difference should be interpreted with caution.
Table 2.3.—Canonical discriminant functions (DF), eigenvalues, canonical correlations, and variance explained for 12 morphometric variables used to investigate morphological variation in the Sierra Madre clade of the *Thomomys umbrinus* complex (Fig. 2.1). Shown are the character loadings on the 2 significant functions for a comparison of the northern Sierra Madre group to nearby *T. umbrinus* (Northern Desert and Central Plateau) and character loadings on 2 of the 3 significant functions for a comparison of the southern Sierra Madre group to nearby *T. umbrinus* (TMVB and Sierra del Nayar), and *T. atrovarius*. The “Sierra Madre” column is a comparison between the northern and southern subgroups within the Sierra Madre group to investigate within-group geographic variation and had only 1 significant DF.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Northern Sierra Madre</th>
<th>Southern Sierra Madre</th>
<th>Sierra Madre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF 1</td>
<td>DF 2</td>
<td>DF 1</td>
</tr>
<tr>
<td>Occipital-nasal length (ONL)</td>
<td>1.99</td>
<td>-0.04</td>
<td>0.82</td>
</tr>
<tr>
<td>Occipital-incisor length (OIL)</td>
<td>-1.39</td>
<td>-0.12</td>
<td>-0.76</td>
</tr>
<tr>
<td>Nasal length (NL)</td>
<td>-0.43</td>
<td>0.62</td>
<td>1.19</td>
</tr>
<tr>
<td>Rostral width (RW)</td>
<td>0.63</td>
<td>0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>Width of interorbital constriction (IOC)</td>
<td>0.90</td>
<td>0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>Zygomatic breadth (ZB)</td>
<td>0.07</td>
<td>-0.81</td>
<td>-0.11</td>
</tr>
<tr>
<td>Cranial width (CW)</td>
<td>-0.13</td>
<td>0.83</td>
<td>0.08</td>
</tr>
<tr>
<td>Mastoid breadth (MB)</td>
<td>-0.19</td>
<td>-0.08</td>
<td>-0.78</td>
</tr>
<tr>
<td>Length of diastema (DIA)</td>
<td>0.52</td>
<td>-0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>Maxillary toothrow length (MTR)</td>
<td>-0.37</td>
<td>0.44</td>
<td>0.36</td>
</tr>
<tr>
<td>Mandible breadth (BM)</td>
<td>-0.60</td>
<td>-0.02</td>
<td>-0.38</td>
</tr>
<tr>
<td>Length of mandible (ML)</td>
<td>-0.57</td>
<td>0.28</td>
<td>-0.33</td>
</tr>
<tr>
<td>Eigenvalues</td>
<td>2.43</td>
<td>1.25</td>
<td>2.09</td>
</tr>
<tr>
<td>Proportion of variance explained</td>
<td>0.66</td>
<td>0.34</td>
<td>0.58</td>
</tr>
<tr>
<td>Canonical correlation</td>
<td>0.84</td>
<td>0.75</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Fig. 2.4.—Distribution of discriminant function scores for 134 specimens of *Thomomys* on the first 2 discriminant functions (DF1 and DF2) based on 12 cranial measurements. Ellipses enclose ≥ 80% of points, disregarding outliers. a) Comparison of specimens from the northern Sierra Madre clade with nearby individuals of the Central Plateau and Northern Desert clades. b) Comparison of specimens from the southern Sierra Madre clade with nearby individuals representing the Trans-Mexico Volcanic Belt clade, the Sierra del Nayar clade, and *T. atrovarius*. Three significant discriminant functions correctly assigned 86.7% of the individuals to the correct group: 86.4% for Sierra Madre individuals, 100% for Sierra del Nayar specimens, 70% for TMVB specimens, and 89.5% for *T. atrovarius* individuals. NL had a relatively high, positive loading on DF 1, and MB and BM had a high positive and negative loading, respectively, on DF 2 (Table 2.3). A plot of the first 2 discriminant functions (Fig. 2.4b) shows the southern Sierra Madre clade to be fairly well separated from the TMVB and Sierra del Nayar groups, although there is more overlap with *T. atrovarius*.

When individuals of the northern Sierra Madre subgroup (currently placed in the subspecies *T. u. chihuahuae* and *T. u. madrensis*) were compared morphometrically to individuals of the southern Sierra Madre subgroup (subspecies *T. u. sheldoni* and *T. u. crassidens*), ANOVA tests showed northern individuals to be significantly larger for ONL ($F_{1,52} = 10.30, P = 0.002$), NL ($F_{1,52} = 6.5, P = 0.014$), and DIA ($F_{1,52} = 6.41, P = 0.014$). Only 1 significant function was generated in the DFA (Wilks’ $\lambda = 0.40, d.f. = 12, P < 0.001$). ONL and
DIA showed high, positive loadings on this axis, whereas ML showed strong, negative loading (Table 2.3). The DFA assigned 90.9% of northern Sierra Madre and 84.6% of southern Sierra Madre individuals to their correct subgroup. All size differences should be interpreted with caution because of phenotypic plasticity inherent in *Thomomys* (Patton and Brylski 1987; Smith and Patton 1988).

### 2.4 DISCUSSION

I employ the biological species concept in this study, recognizing that the life history characteristics of Geomyidae (patchy distributions, small population sizes, and exceptional genetic structure) can make this occasionally problematic (Steinberg and Patton 2000). In such cases, I refer to diagnosable lineages to guide my species designations. Chromosomal differences in pocket gophers, frequently represented by differences in diploid number, often signal reproductive barriers to gene flow (Patton 1985; Patton and Feder 1978). Thus, populations of pocket gophers that represent monophyletic groups and show no differences in diploid number are considered conspecific until future data are available to refute this claim.

My multi-locus analysis of DNA sequence data shows all *T. umbrinus* populations that possess a diploid number of 78 chromosomes to be monophyletic. The 2n = 78 populations comprise 3 genetically distinguishable clades (Northern Desert, Central Plateau, and Trans-Mexico Volcanic Belt; Figs. 2.1 and 2.2) that appear to be separated by barren, rocky habitat that may inhibit dispersal. The discordance between allozyme data presented in Hafner et al. (1987) and newly generated sequence data for localities 8 and 10 (Fig. 2.1) may signal recent or limited gene flow between the Northern Desert and Central Plateau subclades. The positive relationship between range size and within-clade genetic variation in the 2n = 78 clades is consistent with the isolation-by-distance explanation and is supported by the significant, positive relationship shown.
in the Mantel test. The three clades share haplotypes at four of five nuclear loci, which could reflect shared ancestral polymorphisms or could be evidence of limited gene flow. Regardless, the 3 clades together form a monophyletic group and share the same diploid number, so they are considered conspecific. The type locality of *T. umbrinus* (locality 34) lies within the 2n = 78 clade, so this taxon (containing the Northern Desert, Central Plateau, and TMVB subclades) retains the species name *umbrinus*.

Although three genetically distinct clades within the *T. umbrinus* complex share a diploid number of 76, this character is of dubious phylogenetic value because it also is shared with the outgroup, *T. bottae*, and is almost certainly the primitive diploid number within the *Thomomys* subgenus *Megascapheus* (reviewed by Hafner et al. 1983 and Patton 1981). Hafner et al. (2011) suggested that the 2n = 76 clades may be monophyletic, but bootstrap support for monophyly in their summary tree based on mitochondrial and nuclear sequences was moderate (bs = 78). Similarly, the three *T. umbrinus* clades with diploid numbers of 76 are not depicted as monophyletic in this study once nodes with weak branch support are collapsed (Fig. 2.2).

Following the recommendation of Álvarez-Castañeda (2010), Hafner et al. (2011) formally elevated the Coastal Sinaloa clade of *T. umbrinus* (2n = 76) to full species status (as *T. atrovarius*) based on phylogenetic, morphological, and ecological evidence combined with absence of detectable gene flow with populations of pocket gophers from the adjacent Sierra Madre Occidental.

A second 2n = 76 clade was identified in this study from the Sierra del Nayar in northeastern Nayarit. This geographically restricted clade (only 2 known populations) is in close proximity to populations of the Sierra Madre clade (13 km to the north) and a population of *T. atrovarius* (11 km to the southwest). There were no shared haplotypes among the 3 populations
(except for TBO47, which exhibited little variation in the data). Current genetic data support my conclusion that this clade represents a new species of pocket gopher. However, I refrain from formally naming this new species at this time and will present a formal description once all morphological and genetic data have been gathered and analyzed.

The third 2n = 76 clade within the T. umbrinus complex, the Sierra Madre clade (Figs. 2.1 and 2.2), has a long, narrow geographic distribution extending almost the entire length of the Sierra Madre Occidental. Patton and Feder (1978) were the first to suggest, based on allozymic and karyological evidence, that the 2n = 76 Sierra Madre clade of T. umbrinus was potentially genetically isolated from nearby populations of the 2n = 78 Northern Desert clade where the 2 groups come into close contact in northwestern Chihuahua. They reported 2 fixed allelic differences between populations of the 2 clades located only about 10 km apart and separated by no obvious ecological barrier. Patton and Feder (1978) also noted that structural rearrangements of chromosomes resulting in different diploid numbers often are indicative of reproductive incompatibility in pocket gophers (Patton and Yang 1977; Thaeler 1974). They identified 2 genetically divergent clades within the 2n = 76 Sierra Madre group—one in the north and 1 in the south—potentially separated by the Barranca del Cobre in south-central Chihuahua.

Increased sampling in the present study has narrowed the 10 km gap between Northern Desert and Sierra Madre populations reported by Patton and Feder (1978) to approximately 2 km. Although I did not find the animals in contact, my explorations in the area confirmed that there are no obvious ecological or geographical barriers between the 2 clades. I conclude that populations of the Northern Desert and Sierra Madre clades probably come into contact in this area, but the extremely patchy distribution of pocket gophers in this rugged, mountainous region likely prevented me from finding the animals in contact. As reported by multiple authors (e.g.,
Hafner et al. 1983; Patton et al. 1984; Thaeler 1974, 1985), limited interbreeding with little or no genetic introgression can occur between pocket gophers of different species. For example, where $2n = 78$ *T. umbrinus* of the Northern Desert clade meet $2n = 76$ *T. bottae* in Arizona, limited hybridization occurs with no evidence of introgression due to meiotic imbalances that result in male sterility (Patton 1973; Patton and Dingman 1968).

In my study, the absence of shared haplotypes at 7 loci, the strict discordance in genotypes as revealed by assignment tests (Fig. 2.3), and the high level of genetic differentiation (14–18% *Cytb* divergence between individuals only 2 km apart) indicate that the Northern Desert and Sierra Madre clades are genetically, if not reproductively, isolated where they meet in northwestern Chihuahua. Álvarez-Castañeda (2010) recommended elevation of the Sierra Madre clade to full species status as *T. chihuahu[a]e* (sic), but my expanded sampling of populations in the southern Sierra Madre Occidental and the inclusion of specimens from the type locality of *T. u. sheldoni* in Nayarit reveals that the species name *T. sheldoni* Bailey 1915 has nomenclatorial priority within the Sierra Madre clade.

The geographic distribution of *T. sheldoni* spans 10º of latitude (almost 1,000 km) in the Sierra Madre Occidental and consists of north and south genetic subclades. There is a large, >200 km gap between northern and southern Sierra Madre populations where no records of specimens exist. This gap may reflect the actual distribution of the 2 subgroups, but I suspect that it is simply an artifact of poor sampling in this remote, mountainous region of western Durango. Current data show the northern and southern populations to be reciprocally monophyletic, although the population at El Vergel (locality 15 in Fig. 2.1) is only weakly linked with the northern subclade (bs = 48). The 2 subclades show somewhat different cranial morphologies, as northern individuals tend to have larger and longer skulls. I formally recognize these genetic,
morphological, and distributional differences between the northern and southern subclades of *T. sheldoni* by establishing 2 subspecies, *T. s. sheldoni* in the south and *T. s. chihuahuae* in the north. A synonymy of *T. sheldoni* follows, along with comments on distinguishing individuals belonging to *T. sheldoni* from geographically adjacent individuals of *T. umbrinus* and *T. atrovarius*.

*Thomomys sheldoni* Bailey, 1915

Sierra Madre Occidental pocket gopher

(Synonymy under subspecies)

**Geographic range.**—Restricted to the upper elevations (≥ 2000 meters) of the Sierra Madre Occidental from west-central Chihuahua extending southward through western Durango to northeastern Nayarit and western Zacatecas (Sierra Madre clade in Fig. 2.1).

**Description.**—Pelage moderately dense, medium to dark brown on dorsum, occasionally with a faint, slightly darker dorsal stripe. Ventrum often golden or yellowish brown with a slightly lighter wash of golden brown on the sides. One pair of pectoral mammae in females.

Diploid number is 76.

**Comments.**—Anderson (1972) placed part of *T. u. sheldoni* in synonymy under *T. u. madrensis* in his examination of *Thomomys* in Chihuahua, but did not include specimens from the type locality of *T. u. sheldoni* (in northeastern Nayarit) in his investigation. Because of Anderson’s action, *T. u. sheldoni* is listed as a junior synonym in recent taxonomic references (Patton 2005), but this name should be considered available.

*Thomomys sheldoni chihuahuae* Nelson and Goldman, 1934

*T. u. chihuahuae* Nelson and Goldman, 1934:114. Type locality: “Sierra Madre, about 65 miles east of Batopilas, Chihuahua, Mexico (altitude 7,000 feet).”
**Geographic range.**—Restricted to high elevation (≥ 2,000 m) habitats of the Sierra Madre Occidental in Chihuahua, north of approximately 25º N. Individuals previously assigned to *T. u. madrensis* found south of 2 km south of Colonia Garcia, Chihuahua (south of approximately 29.95º N) are herein recognized as *T. s. chihuahuae*. This taxon does not include individuals of *T. u. chihuahuae* east of the Sierra Madre Occidental (below 2,000 m) in Chihuahua (see Anderson 1972) or those in Durango (see Baker and Greer 1962).

**Comments.**—Hafner et al. (2011) assigned all specimens of *T. u. eximius* except those from the type locality to *T. atrovarius* based on a combination of genetic and morphometric evidence. In that study, morphometric evidence suggested that specimens from the type locality in extreme northeastern Sinaloa belonged to the Sierra Madre clade of *T. umbrinus*, which I now recognize as *T. sheldoni*. The taxonomic placement of specimens from the type locality of *T. u. eximius* is problematic because the exact location of the type locality is unknown (Goldman 1951:251) and therefore could not be resampled. Attempts to extract useful DNA from 114 year-old study skins of 2 paratype specimens were unsuccessful. However, ancient DNA extracted from study skins of specimens collected more recently from 2 localities (18 km NNE Choix and 1.5 mi. ENE El Cajon) near the presumed location of the type locality show them to be *T. atrovarius*. Accordingly, I tentatively regard *T. u. eximius* as a subjective junior synonym of *T. atrovarius* until such time as evidence emerges to support or refute this decision.

*T. s. chihuahuae* likely comes into contact with *T. u. madrensis* (Northern Desert clade; Fig. 2.1) near the town of Colonia Garcia in northwestern Chihuahua. *T. umbrinus* in this region has 2n = 78 chromosomes, whereas *T. sheldoni* has a diploid number of 76. *T. s. chihuahuae* individuals are on average larger than individuals of the Northern Desert form of *T. umbrinus*. Characters generally useful for distinguishing *T. s. chihuahuae* from nearby *T. umbrinus*
individuals are (all dimensions in mm) total body length > 180, ONL > 34.2, OIL > 34.7, and MTR > 7.2. The closest known population of the Central Plateau clade (locality 14 in Fig. 2.1) is located ca. 17 km east of the Sierra Madre population at locality 15 (Fig. 2.1). Because these populations live in different habitats at different elevations (locality 14 is in high desert habitat at 1,730 m, whereas locality 15 is in pine-oak forest at 2,712 m), it is unlikely that these taxa will be found in contact. *T. umbrinus* individuals of the Central Plateau clade have 2n = 78 chromosomes.

Specimens of *T. s. chihuahuae* from El Vergel, Chihuahua (locality 15 in Fig. 2.1) appear to be intergrades between this subspecies and *T. s. sheldoni*. Hafner et al. (1987) assigned specimens from this locality to the southern Sierra Madre clade (*T. s. sheldoni*) based on allozyme data, but my multi-locus analyses have them either weakly linked with the northern Sierra Madre clade (ML) or basal to the Sierra Madre clade (BI). For the time being, I have assigned specimens from El Vergel to *T. s. chihuahuae* based on their geographic location, while recognizing that they are most likely intergrades between the 2 subspecies of *T. sheldoni*.

*Thomomys sheldoni sheldoni* Bailey, 1915

*Thomomys sheldoni* Bailey, 1915:93. Type locality: “Santa Teresa (6,800 feet altitude), Tepic, Mexico.” Type specimen adult male, skin and skull, U.S. National Museum, Biological Survey collection (USNM) number 90819, collected 10 August 1897 by E. W. Nelson and E. A. Goldman, collectors’ number 11443.


*T. u. crassidens* Nelson and Goldman, 1934:113. Type locality “Sierra de Valparaiso, western Zacatecas, Mexico (altitude 8,700 feet).”

Geographic range.—*T. s. sheldoni* appears to be restricted to the high elevation (≥ 2,000 m)
habitats in the predominantly pine-oak forests of the Sierra Madre Occidental in western Durango, northeastern Nayarit, and western Zacatecas. Specimens of Thomomys from the Sierra Madre Occidental of Durango previously recognized as *T. umbrinus chihuahuae* are now recognized as *T. s. sheldoni*. This taxon does not include individuals recognized by Matson and Baker (1986) as *T. umbrinus sheldoni* from the vicinity of Monte Escobedo, Zacatecas. Individuals formerly assigned to *T. u. crassidens* from the vicinity of Chalchihuites, Zacatecas (Matson and Baker 1986) were assigned to *T. s. sheldoni* in this study based on cranial morphology (fresh tissues were not available for molecular analysis). Ongoing ancient DNA analyses hopefully will elucidate the phylogenetic position of these specimens, but until then, they are provisionally assigned to *T. s. sheldoni*.

**Comments.**—Populations of *T. s. sheldoni* in northeastern Nayarit are in close proximity (within 13 km) to populations of a genetically distinct clade of *Thomomys* (also 2n = 76) in the Sierra del Nayar. Specimens of *T. s. sheldoni* are, on average, larger than specimens of the Sierra del Nayar form in all cranial dimensions (Fig. 2.4b), although this distinction may not hold once larger samples of the Sierra del Nayar form become available. Populations of *T. s. sheldoni* in northeastern Nayarit also occur within 29 km of populations of *T. atrovarius*. Characters useful for distinguishing specimens of *T. atrovarius* from specimens of other *Thomomys* species in Mexico were described by Hafner et al. (2011).
CHAPTER 3

THOMOMYS NAYARENSIS, A NEW SPECIES OF POCKET GOPHER FROM THE SIERRA DEL NAYAR, NAYARIT, MEXICO

3.1 INTRODUCTION

The high biological diversity of the Mexican state of Nayarit no doubt is influenced by the extreme topographical complexity of this region. Situated along the Pacific Coast, this relatively small state (27,815 km²) contains both broad coastal plains and rugged mountains exceeding 2,000 m elevation in the southern versant of the Sierra Madre Occidental. Researchers have recognized that this state “…occupies an important position in understanding patterns of mammalian distribution and problems of taxonomy in western Mexico, especially for small mammals of limited vagility.” (Carleton et al. 1982:1)

Smooth-toothed pocket gophers in the genus Thomomys (Geomyidae) are fossorial rodents with both limited vagility and problematic taxonomy. Patchily distributed populations with high rates of molecular evolution (Spradling et al. 2001) have resulted in geomyid populations of the same species that are as genetically divergent as other well-characterized species of mammals (Hafner et al. 1983; Patton and Yang 1977). This high level of genetic divergence, coupled with conserved morphology and extremely variable pelage color and body size, has made recognition of pocket gopher species problematic regardless of one’s species concept. Despite these difficulties, my studies of relationships among geomyid populations using a combination of nuclear and mitochondrial DNA sequence data, morphology, and comparative cytogenetics have allowed me to identify diagnosable and genetically isolated clades within Thomomys, which I use, coupled with the biological species concept, as my operational definition of “species” (Hafner et al. 2005, 2011; Chapter 2).
In this chapter I describe a new species of pocket gopher from the Sierra del Nayar of northeastern Nayarit. This isolated lineage was discovered in the course of a larger study of the *Thomomys umbrinus* complex. First reported as *T. u. sheldoni* from near Santa Teresa, Nayarit by Hafner et al. (2011), the population (referred to as the “Santa Teresa clade” in that study) showed an average of nearly 16% cytochrome *b* (*Cytb*) divergence from 3 nearby clades of *Thomomys* and had an unresolved phylogenetic relationship with these clades. With the addition of new samples from the Sierra del Nayar and inclusion of data generated by analyses of ancient DNA and morphology, I am now able to describe the “Santa Teresa clade” as a new species and provide a better understanding of the relationships between this species and other species of *Thomomys* in Mexico.

3.2 MATERIALS AND METHODS

**Sampling, karyotyping, and DNA sequencing.**—The region of Sierra del Nayar shown in Fig. 3.1 was sampled for pocket gophers in 2009, 2011, and 2012. Most populations of *Thomomys* in this region were small and difficult to locate, but I was able to collect eight specimens of the new species. The four specimens collected in 2011 were karyotyped using the post-mortem technique developed by Hafner and Sandquist (1989). Specimens were collected using trapping methods approved by the American Society of Mammalogists (Sikes et al. 2011). Vouchers were prepared as skin-plus-skeleton specimens (Hafner et al. 1984) and deposited in the Louisiana State University Museum of Natural Science (LSUMZ) or the Colección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México (CNMA).

In addition to eight representatives of the new species, my phylogenetic analysis included four specimens of the recently resurrected species *T. sheldoni* (Chapter 2), four specimens of *T. umbrinus* from the nearby Trans-Mexico Volcanic Belt clade (TMVB)—see
Fig. 3.1.—Distribution of the Thomomys umbrinus species group in Mexico and southwestern United States and close-up of the Sierra del Nayar region showing the location of samples used in the genetic analyses (Appendix 3.1). Thomomys umbrinus clades were originally defined by Hafner et al. (1987) based on allozyme and chromosomal data. Thomomys atrovarius was elevated to species status by Hafner et al. (2011) and T. sheldoni was elevated to species status in Chapter 2.

Chapter 2), three specimens of T. atrovarius from the Pacific lowlands (Hafner et al. 2011), and two specimens of T. bottae from northern Sinaloa. One specimen each of T. mazama and T. talpoides were included to represent the subgenus Thomomys, and one specimen of Orthogeomys hispidus was included in the analysis as an outgroup. Collection localities are listed in Appendix 3.1 and mapped in Fig. 3.1.

DNA sequences were obtained from three mitochondrial loci: Cytb (1,140 base pairs [bp]), 12S rRNA (12S; 865 bp), and cytochrome oxidase I (COI; 1,545 bp). Five nuclear genes
also were sequenced, including the 5’ end of exon 1 of the single-copy interphotoreceptor retinoid binding protein (IRBP; 1,272 bp), the growth hormone receptor gene (GHR; 832 bp), recombination activating protein I (Rag1; 1,293 bp), the mast cell growth factor protein (MGF; 727 bp), and one anonymous locus (TBO47 from Belfiore et al. 2008; 601 bp). DNA amplification and sequencing protocols may be found in Chapter 2 of this dissertation and a list of primers and their annealing temperatures are available in Appendix 2.2.

Pocket gophers from 5 localities were included in my analysis of ancient DNA. Skin clips were obtained from museum study skins collected between 1955 and 1977 and amplified for a fragment of Cytb. Amplification protocols, sequencing protocols, and primer information for these analyses are available in Hafner et al. (2011). All DNA sequences used in this study are deposited in GenBank (Appendix 3.2) with the exception of 7 ancient DNA sequences that did not fit the minimum length requirements of GenBank (Appendix 3.3).

Phylogenetic analyses. — The 12S alignment was explored in the program GBlocks (Castresana 2000) and uninformative gaps and indels were removed. Analyses based on Bayesian Inference (BI) were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and maximum likelihood (ML) analyses were implemented in RaxML 7.3.0 (Stamatakis 2006) via the CIPRES Gateway (Miller et al. 2010). We evaluated the most appropriate models for each gene in MrModelTest 2.4 (Nylander 2004), which provides models appropriate for both BI and ML analyses. We selected the best model using the Akaike Information Criterion (Posada and Buckley 2004).

For both sets of phylogenetic analyses (BI and ML), sequences were concatenated and then partitioned by gene. For the BI analysis, model parameters were treated as unknown variables with uniform priors. The GTR+I+G model was used for Cytb, COI, 12S, and IRBP, the
HKY+I model for *GHR, MGF*, and *Rag1*, and the HKY model for *TBO47*. Two independent runs were initiated with random starting trees, an initial melting point of 0.25, and run for at least $9 \times 10^6$ generations with 4 incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck and Ronquist 2001) and sampled every 100 generations. Convergence and stationarity were assessed using Tracer v1.5 (Rambaut and Drummond 2007). Trees generated before stationarity of log-likelihood scores was reached were discarded. Clade support was assessed using Bayesian posterior probabilities. ML gene-partitioned analyses were run for 1,000 bootstraps, using the GTRCAT model for the bootstrapping phase in RaxML and the GTRGAMMA model for the tree inference phase.

**Morphometric analysis.**—Visual inspection revealed the auditory meatus to be a potentially diagnostic feature for distinguishing the new species from its congeners (Fig. 3.2). This character was used to augment the data on cranial morphology for Mexican species of *Thomomys* presented in Chapter 2. The maximum interior diameter of the opening of the auditory meatus was measured in the anterior-posterior plane (“width of auditory meatus”) and dorsal-ventral plane (“height of auditory meatus”). Because the auditory meatus was too delicate to measure with hand-held calipers, width and height of the auditory meatus and occipital-nasal length were recorded from cranial photographs of specimens of *T. sheldoni, T. atrovarius*, and the new species in tpsDig 2.16 (Rohlf 2010). A 1-way ANOVA was performed on these 2 measurements of the auditory meatus (standardized by occipital-nasal length), and post hoc analyses of the ANOVAs were assessed with Tukey’s HSD.

Cranial morphometric data from Chapter 2 were reanalyzed to focus solely on the 3 *Thomomys* species in the Sierra del Nayar region. Because geomyids often show extreme sexual
Fig. 3.2.—Dorsal, ventral, and lateral views of cranium and lateral view of mandible of holotype of *Thomomys nayarensis* (LSUMZ 36794). Two measurements of the auditory meatus discussed in the text (H = height of the auditory meatus and W = width of the auditory meatus) are shown.

dimorphism in morphology, only adult female skulls were measured for 12 cranial characters: cranial width (CW), diastema length (DIA), width of interorbital constriction (IOC), mastoid breadth (MB), length of maxillary tooth row (MTR), nasal length (NL), occipital-nasal length (ONL), occipital-incisor length (OIL), rostral width (RW), zygomatic breadth (ZB), breadth of mandible (BM), and mandible length (ML). Twenty-two specimens of *T. atrovarius*, 30 of *T. sheldoni*, and 9 of the new species were included in the reanalysis of the cranial data.

All statistical analyses of the morphometric data from Chapter 2 were conducted using SPSS 19 (IBM, Armonk, New York). Data were explored for normality and transformed ($\bar{X} = 0$, $SD = 1$). A multivariate analysis of variance (MANOVA) was used to test the null hypothesis of
no significant difference between \textit{a priori} groups. A post hoc analysis of the MANOVA was assessed with Tukey’s HSD. A principal components analysis (PCA) was performed using a varimax rotation to reduce the 12 variables and explore the dimensionality of the data. Direct discriminant function analysis (DFA) was performed to generate discriminant functions to predict group membership and evaluate if individuals could be properly assigned to their \textit{a priori} genetic groups.

3.3 RESULTS

Phylogenetic analyses.—Inspection of individual gene trees (not shown) revealed that individuals representing the new species formed a monophyletic group in the BI and ML analyses of \textit{Cytb}, \textit{12S}, \textit{COI}, \textit{IRBP}, and \textit{RAG1} and in the ML analysis of \textit{MGF}. After weakly supported nodes were collapsed (bootstrap [bs] < 85\%, Bayesian posterior probabilities [pp] < 0.95), \textit{T. sheldoni}, \textit{T. umbrinus}, \textit{T. atrovarius}, \textit{T. bottae}, and the new species (all representatives of the \textit{Thomomys} subgenus \textit{Megascapheus}) formed a polytomy when the 3 mitochondrial genes were analyzed together. Individuals of the new species shared no haplotypes with the other genetic groups for any of the genes except \textit{TBO}, which had only 5 unique haplotypes for the dataset and was phylogenetically uninformative.

The \textit{Cytb} gene had 323 parsimony informative sites, and 3 genes (\textit{Cytb}, \textit{12S}, and \textit{IRBP}) together had 37 nucleotide substitutions that distinguished individuals of the new species from other representatives of \textit{Thomomys} in the alignment (Table 3.1). Prior to nodal collapse, the \textit{Cytb} analyses revealed a weak sister relationship between the new species and \textit{T. umbrinus} individuals from the TMVB (pp = 0.75, bs = 59). The \textit{COI} gene showed a conflicting, but equally weak, sister relationship between the new species and individuals of \textit{T. sheldoni} from the Sierra Madre
Table 3.1.—Nucleotide substitutions at the cytochrome b (Cytb), 12S rRNA (12S), and interphotoreceptor retinoid binding protein (IRBP) loci that distinguish the new species, *Thomomys nayarensis*, from *T. umbrinus*, *T. sheldoni*, and *T. atrovarius*. Numbers indicate the nucleotide position of the change. The base to the left of each arrow is present in *T. umbrinus*, *T. sheldoni*, and *T. atrovarius* and the base to the right is diagnostic for *T. nayarensis*. Ambiguity codes (“K” for G/T, “R” for A/G, “W” for A/T, and “Y” for C/T) indicate nucleotide positions at which *T. umbrinus*, *T. sheldoni*, and *T. atrovarius* may not share the same base.

<table>
<thead>
<tr>
<th><em>Cytb</em> position (1,140 bp)</th>
<th>Base change</th>
<th><em>12S</em> position (865 bp)</th>
<th>Base change</th>
<th><em>IRBP</em> position (1,272 bp)</th>
<th>Base change</th>
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<td>1140</td>
<td>A → G</td>
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Fig. 3.3.—Genetic relationships among species of Thomomys in and near the Sierra del Nayar region of Nayarit, Mexico, based on gene-partitioned Bayesian and maximum likelihood analyses of 8 genes (8,275 bp). The Bayesian tree topology is shown. Black circles indicate well-supported nodes, white circles are nodes with high posterior probabilities but weaker bootstrap support, and nodes with weak support (posterior probability < 0.95 or bootstrap support < 80%) are collapsed. Numbers before locality names refer to the map (Fig. 3.1), and full locality information is listed in Appendix 3.1. Diploid numbers are indicated on major branches of the tree, and average percent Cytb sequence divergence values are shown for 2 major nodes. Outgroups include 1 specimen of Orthogeomys hispidus and 2 individuals representing the subgenus Thomomys (T. mazama and T. talpoides). Scale bar represents the estimated number of substitutions per site. Asterisks next to selected localities indicate ancient DNA samples.

Occidental (pp = 0.63, bs = 57), and this latter relationship was supported strongly by the IRBP gene (pp = 0.95, bs = 86).

The full dataset (8,275 bp concatenated and partitioned by gene) revealed an unresolved polytomy for T. sheldoni, T. umbrinus, and the new species (Fig. 3.3). Prior to nodal collapse, there was a weakly supported sister relationship between individuals of the new species and individuals of T. umbrinus from the TMVB (pp = 0.77, bs = 61).
Genetic Distances.—Average Kimura 2-parameter genetic distances at \textit{Cytb} for \textit{Thomomys} specimens from the Sierra del Nayar region ranged from 14\% (between \textit{T. sheldoni} and the new species) to 18.5\% (between \textit{T. atrovarius} and the new species). Average genetic distance between individuals of the new species and \textit{T. umbrinus} individuals from the TMVB was 15.4\%. Individuals of \textit{T. sheldoni} and the new species captured only 13 km apart ranged in pair-wise genetic distances from 15.6\% to 18.5\%.

Morphometric analysis.—Individuals of \textit{T. sheldoni} (\(n = 9\)) were significantly larger than individuals of the new species (\(n = 9\)) and \textit{T. atrovarius} (\(n = 5\)) for both width and height of the auditory meatus (\(F_{2, 20} = 17.69, P < 0.001\) and \(F_{2, 20} = 16.32, P < 0.001\), respectively; Table 3.2). Tukey’s HSD post-hoc test did not reveal a significant difference between \textit{T. atrovarius} and the new species for either dimension of the auditory meatus (Table 3.2).

Exploration of the cranial morphometric data revealed 2 variables (IOC and CW) that were non-normally distributed, but after removal of extreme outliers, only IOC remained non-normal. IOC was removed from the MANOVA but was retained for the PCA and DFA because these analyses are robust to deviations from normality not caused by outliers (Tabachnick and Fidell 1996). The MANOVA revealed significant differences between the 3 species (Pillai’s Trace = 1.32, \(F_{24,84} = 6.86\); Wilks’ Lambda = 0.11, \(F_{24,82} = 6.7\); Hotelling’s Trace = 3.92, \(F_{24,80} = 6.54\); \(P < 0.0001\) for all 3 statistics). Post hoc tests of the MANOVA revealed the new species of \textit{Thomomys} to be significantly smaller than \textit{T. sheldoni} and \textit{T. atrovarius} for ONL, OIL, NL, RW, CW, MB, DIA, MTR, BM, and ML. The new species was significantly smaller than \textit{T. sheldoni} for ZB.

The PCA revealed 3 components (eigenvalues > 1) that explained 79.6\% of the variation in the data. After a varimax rotation, ONL, OIL, NL, MB, DIA, BM, and ML loaded heavily
Table 3.2.—Means and standard errors (with ranges in parentheses) of auditory meatus width (maximum interior diameter of the opening of the auditory meatus measured in the anterior-posterior plane; Fig. 3.2) and auditory meatus height (maximum interior diameter of the opening of the auditory meatus measured in the dorsal-ventral plane) in 3 species of *Thomomys*. Means that share a superscript are not significantly different from each other (Tukey’s HSD, $P < 0.05$).

<table>
<thead>
<tr>
<th>Species</th>
<th>Width of auditory meatus</th>
<th>Height of auditory meatus</th>
<th>Occipital-nasal length</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. nayarensis</em></td>
<td>1.13 ± 0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.29 ± 0.073&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>(n = 9)</td>
<td>(0.9 – 1.3)</td>
<td>(0.8 – 1.5)</td>
<td>(29.0 – 36.3)</td>
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<tr>
<td><em>T. atrovarius</em></td>
<td>1.26 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.84 ± 0.057&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(1.0 – 1.4)</td>
<td>(1.1 – 1.4)</td>
<td>(32.7 – 35.9)</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td>1.79 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.39 ± 0.053&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>(n = 9)</td>
<td>(1.5 – 2.3)</td>
<td>(1.6 – 2.2)</td>
<td>(32.8 – 36.9)</td>
</tr>
</tbody>
</table>

 (>0.75) on PC 1, IOC loaded heavily on PC 2, and ZB loaded heavily on PC 3. Visual exploration of the principal component scores did not reveal any clear pattern (Fig. 3.4a).

Discriminant function analyses resulted in 2 significant functions that assigned 91.2% of the individuals to the correct species group (85% of *T. atrovarius*, 92.9% of *T. sheldoni*, and 100% of the new species). The new species of *Thomomys* could be distinguished from *T. atrovarius* and *T. sheldoni* along the 1<sup>st</sup> DF axis (Fig. 3.4b). NL and ONL had high, positive DF coefficients on DF 1 (1.03 and 0.90, respectively). OIL had a high negative coefficient (-1.27) and MB had a positive coefficient (1.35) on DF 2.

Chromosomal analysis.—Examination of chromosomal preparations from 4 specimens of the new species captured at locality 9 (Fig. 3.1) showed them to have a diploid number of 76 and a fundamental number of 146 (mostly biarmed chromosomes).

3.4 DISCUSSION

Nayarit is home to interesting, and often unexplained, patterns of mammalian diversity (Carleton et al. 1982, 1999; Rogers and Engstrom 1992; Rogers and Vance 2005; Schmidly and Schroete 1974; Tiemann-Boege et al. 2000). This diversity, and accompanying high levels of
endemism, extends throughout the Mexican highlands for many vertebrate groups (Bryson et al. 2011 and included references; García 2006). Vicariance events during the Neogene and subsequent climate change in the Quaternary are considered to be the primary causal forces shaping this diversity (Ferrusquía-Villafranca and González-Guzmán 2005).

Patterns of the evolutionary relationships among *Thomomys* species in Nayarit are complicated and not fully resolved by this study. My argument for species status of the genetically divergent populations of *Thomomys* in northeastern Nayarit parallels the argument used in Chapter 2 for recognition of *T. sheldoni*. First, the Nayarit populations are monophyletic and show as much as 18.5% *Cytb* divergence from nearby populations of *Thomomys* (Fig. 3.3). Second, monophyly of this group is supported by multiple nuclear and mitochondrial genes. Finally, there appears to be no gene flow (no shared haplotypes at 7 of 8 loci examined) between
the new species and populations of *T. sheldoni* located only 13 km away. Populations of *T. sheldoni* and the next species are found on opposite sides of a narrow constriction of tableland (<400 m wide in places) that extends for over two km and has steep, 100 m drops on either side. The constriction itself is cut in several places by steep gorges. I do not know if the heavily eroded soils along the top of this constriction are deep enough to support gopher populations, but I saw no evidence of gophers in this region in 2011 and 2012.

The new species appears to be more closely related to *T. sheldoni* than to *T. atrovarius* based on molecular evidence (Fig. 3.3). In addition, the new species and many populations of *T. sheldoni* have high chromosome fundamental numbers (FN ≥ 138), whereas no population of *T. atrovarius* karyotyped to date has FN > 132. Molecular data from this study were insufficient to resolve relationships among *T. sheldoni*, *T. umbrinus*, and the new species (Fig. 3.3). The fact that *T. sheldoni* and the new species share diploid numbers (2n = 76) to the exclusion of *T. umbrinus* (2n = 78) is of dubious phylogenetic significance because 2n = 76 is the presumed ancestral diploid number for the subgenus *Megascapheus* (Hafner et al. 1983; Patton 1981).

Polytomies are especially difficult to resolve in a recent and rapid phylogenetic radiation, such as that postulated for pocket gophers by Spradling et al. (2004). Species tree analyses by Belfiore at al. (2008) showed the *Thomomys* radiation to be relatively recent; e.g., the split between *T. sheldoni* and *T. atrovarius* (referred to in that publication as *T. u. chihuahuae* and *T. u. atrovarius*, respectively) was dated between 0.15 and 0.88 Ma using fossil calibrations. As molecular techniques and analyses become more refined, such recent and rapid radiations may one day be fully resolvable.

Probably because of their fossorial habits, pocket gophers show extreme morphological conservatism, yet populations, even conspecific populations, often vary widely in terms of body
size and pelage coloration and texture. These aspects of pocket gopher morphology often make it difficult to identify features that can be used reliably to distinguish closely related species. Although my morphometric analysis shows all known specimens of the new species to be smaller in body size and cranial dimensions than nearby populations of *T. sheldoni*, body size has been shown to be a notoriously unreliable phylogenetic character in pocket gophers (Hafner et al. 2008; Patton and Brylski 1987; Smith and Patton 1988). The 2 new morphological characters used in this study (width and height of the auditory meatus; Fig. 3.2 and Table 3.2) may assist researchers in distinguishing the new species from *T. sheldoni*, and characters useful for distinguishing the new species from *T. atrovarius* were described by Hafner et al. (2011).

*Thomomys nayarensis*, new species

Nayar pocket gopher

**Holotype.**—Adult male; skin, skull, partial skeleton; Louisiana State University Museum of Natural Science, LSUMZ 36794; from Mexico: Nayarit; 8.5 km N, 7 km W Mesa del Nayar (formerly listed by Hafner et al. [2011] as “22 km S, 3 km E Santa Teresa”), 2,200 m (22.290, -104.721); collected 15 January 2011. Original number Mark S. Hafner 1852; Tissue (kidney and liver) deposited in the Louisiana State University Museum of Natural Science Genetic Resources Collection; karyotype available upon request. Other specimens in the type series include 2 males (LSUMZ 36750, 36797) and 4 females (LSUMZ 36751, 36752, 36795, 36796).

**Distribution.**—Known only from 2 localities in the Sierra del Nayar near the town of Mesa del Nayar (El Nayar municipality) in northeastern Nayarit. Known elevational range 1,290 – 2,200 m.

**Diagnosis.**—A medium-size pocket gopher (total length 168 – 210 mm in adults), medium brown on the dorsum and golden/yellowish brown on the ventrum, with a slightly
lighter golden wash on the sides. A few individuals from the type locality had an ochraceus wash on the cheeks and others had light grey flecks in the pelage. *T. nayarensis* is a member of the *Thomomys umbrinus* species group and is smaller than the other 3 members of this group in northeastern Nayarit (*T. sheldoni, T. umbrinus*, and *T. atrovarius*). Known populations of *T. nayarensis* are located near populations of *T. sheldoni* (13 km distant) and *T. atrovarius* (11 km distant), but molecular data show no evidence of gene flow among these 3 species. *T. nayarensis* has a diploid number of 2n = 76, which it shares with *T. sheldoni* and *T. atrovarius*, but not *T. umbrinus* (2n = 78). The auditory meatus of *T. nayarensis* is significantly shorter and narrower that that of *T. sheldoni* (Fig. 3.2 and Table 3.2).

**Etymology.**—The specific epithet *nayarensis* refers to the Sierra del Nayar region of the Sierra Madre Occidental to which *T. nayarensis* appears to be endemic. The name Nayar comes from a 16th century leader and local hero, El Rey Nayar. The Indian tribe in this region, the Cora, and the indigenous language they speak also are known as *Nayeeri* or *Na’ayarij* (López et al. 2010). The roughly 13 km gap that separates *T. nayarensis* from nearby populations of *T. sheldoni* also marks the approximate boundary between 2 mutually unintelligible dialects of the Cora language, Cora del Nayar to the east and Cora Santa Teresa to the west (Lewis 2009).

**Key to the Thomomys from Sierra del Nayar, Nayarit**

1. Dark brown dorsally. Sides of body either same color as dorsum or infused with slight grayish wash. Ventrum similar to side coloration, occasionally with a buffy tint ..... *T. atrovarius*
Medium to dark brown dorsally. Sides of body usually slightly lighter than dorsum or infused with a golden or yellowish (but not grayish) wash. Ventrum with a golden brown wash .......................................................... 2

2. Maximum interior diameter of the opening of the auditory meatus measured in the anterior-posterior plane < 1.6 mm and maximum interior diameter of the opening of the auditory meatus measured in the dorsal-ventral plane < 1.5 mm. Total length of adults usually < 180 mm, but may range up to 210 mm. May have ochraceus wash on cheeks extending to just behind forelegs or gray flecks on dorsum. May occur below 2,000 m elevation .......................................................... \( T. \) \( nayarensis \)

Maximum interior diameter of the opening of the auditory meatus measured in the anterior-posterior plane \( \geq \) 1.6 mm and maximum interior diameter of the opening of the auditory meatus measured in the dorsal-ventral plane \( \geq \) 1.5 mm. Total length of adults \( \geq \) 180 mm. No ochraceus wash on cheeks or gray flecks on dorsum. Only known from above 2,000 m elevation .......................................................... \( T. \) \( sheldoni \)
CHAPTER 4
EVOLUTION AND PHYLOGEOGRAPHY OF THE THOMOMYS UMBRINUS SPECIES COMPLEX (RODENTIA: GEOMYIDAE)

4.1 INTRODUCTION

The term “subspecies,” both as a concept and as an operational unit, has been problematic for systematists since its introduction in the mid-1800s. A subspecies can be viewed as either a unit of classification to describe geographical variation within a species or as an evolving lineage potentially on a trajectory towards speciation (Lidicker 1962). Although these definitions are not mutually exclusive, systematists rarely publish explicit statements about their rationale for naming subspecies. For many taxa, morphological variation may be indicative of incipient speciation (Alexander and Breden 2004; Zimmerman et al. 1978). In others, morphological differences that define subspecies may not coincide with genetic breaks within the species (Conroy and Cook 2000; Conroy and Neuwald 2008). Teasing apart simple phenotypic plasticity from evolutionarily meaningful differences, and correlating genetic diversity with morphological variation is important for a greater understanding of biodiversity (Ramey et al. 2005; Thorpe 1987). The advent of modern genetic techniques provides a way to compare genetic and morphological discontinuities within a species, thereby permitting informed decisions as to what can and should be considered a subspecies, and why.

Taxonomy of the smooth-toothed pocket gophers, Thomomys, has long been fraught with conflict over species and subspecies boundaries. Documented hybridization between T. umbrinus and T. bottae in southern Arizona (Hoffmeister 1969; Patton and Dingman 1968) led Hall (1981) to consider T. umbrinus and T. bottae conspecific and list >200 subspecies of T. umbrinus, largely based on pelage, body size, or other exomorphological differences. Although T. umbrinus and T. bottae are now recognized as separate species, there is still much work to be done re-
evaluating the 18 currently recognized subspecies of *T. umbrinus* (previously 25 subspecies; Patton 2005) and 133 subspecies of *T. bottae*.

This report focuses on the *T. umbrinus* complex, which has undergone major taxonomic revisions over the past several years (Álvarez-Castañeda 2010; Hafner et al. 2011; see also Chapters 2 and 3 in this dissertation). Recent re-evaluations of the genetic clades within *T. umbrinus* originally defined by Patton and Feder (1978) and Hafner et al. (1987) have resulted in the elevation of 2 of these clades to species status: a Pacific coast species, *T. atrovarius* (Hafner et al. 2011), and a Sierra Madre Occidental species, *T. sheldoni* (Chapter 2). A third species, endemic to the Sierra del Nayar in northeastern Nayarit, was also recently discovered and formally described as *T. nayarensis* (see Chapter 3). All 3 of these recently described species have a diploid number (2n) of 76 chromosomes, which is believed to be the primitive diploid number in the *Thomomys* subgenus *Megascapheus* (Hafner et al. 1983; Patton 1980) to which the *T. umbrinus* complex belongs.

Recognition of *T. atrovarius*, *T. sheldoni*, and *T. nayarensis* from within what was traditionally known as *T. umbrinus* leaves the nominal species (*T. umbrinus* sensu stricto) as the only member of the *T. umbrinus* species complex in need of taxonomic revision. *T. umbrinus* sensu stricto (hereafter *T. umbrinus*) currently contains 3 well-defined genetic clades divided into 18 subspecies, all with the derived diploid number of 2n = 78. These clades were defined by Hafner et al. (1987) and in Chapter 2 as the Northern Desert clade, which ranges from southwestern New Mexico and southeastern Arizona into northeastern Sonora and extreme northwestern Chihuahua; the Central Plateau clade distributed from central Chihuahua into north-central Durango; and the Trans-Mexico Volcanic Belt (TMVB) clade distributed from south-central Durango southward into Veracruz (Fig. 4.1). Despite the high degree of genetic
Fig. 4.1.—Distribution of the *Thomomys umbrinus* species complex in Mexico and southwestern United States. *T. atrovarius*, *T. sheldoni*, and *T. nayarensis* are recently elevated species within the complex. The 3 clades within *T. umbrinus* were first characterized based on allozymes and karyotypic data by Hafner et al. (1987) and later redefined in Chapter 2 using multi-locus genetic analyses. Black circles indicate locations of samples used in the genetic analyses; white circles indicate samples used in the morphometric analyses, and gray circles indicate localities used in both analyses. Gray shading and italicized names show the distribution of currently recognized subspecies of *T. umbrinus*. Numbered localities refer to those used in the multi-locus genetic analyses. Locality information is given in Appendix 4.1.
differentiation among these 3 clades (10–21% cytochrome b [Cytb] divergence; Chapter 2), they
were treated as a single species in Chapter 2 because of shared haplotypes suggestive of gene
flow among the clades and a shared, derived diploid number of 2n = 78.

Here we use a combination of multi-locus genetics, allozymes, and morphology to
confirm the species status of *T. umbrinus* and to resolve explicitly defined subspecies boundaries
within the species. A thorough understanding of relationships within this geographically
widespread species permits a large-scale analysis of the phylogeographical history of all 4
members of the *T. umbrinus* species complex in Mexico.

4.2 MATERIALS AND METHODS

Between 2006 and 2012, 124 specimens of *Thomomys* (50 *T. umbrinus*, 32 *T. sheldoni*,
21 *T. atrovarius*, 13 *T. bottae*, and 8 *T. nayarensis*) were collected using standard trapping
methods approved by the American Society of Mammalogists (Sikes et al. 2011). Selected
individuals from most localities were karyotyped in the field using the postmortem technique of
Hafner and Sandquist (1989) to verify diploid numbers. Vouchers were prepared as skin-plus-
skeleton specimens (Hafner et al. 1984) and deposited in the Louisiana State University Museum
of Natural Science (LSUMZ) or the Colección Nacional de Mamíferos, Instituto de Biología,
Universidad Nacional Autónoma de México (CNMA). Frozen tissues from an additional 90
*Thomomys* individuals were obtained from museum tissue collections.

For molecular analyses, 37 specimens of *T. umbrinus*, 4 of *T. sheldoni*, 2 *T. nayarensis*, 3
*T. atrovarius*, and 2 specimens of *T. bottae* were sequenced for 8 genes. Two representatives of
the subgenus *Thomomys* (1 each of *T. mazama* and *T. talpoides*) and 1 specimen of *Orthogeomys
hispidus* were included as outgroups. Collection localities are listed in Appendix 4.1 and mapped
in Fig. 4.1.
DNA sequences were obtained from 3 mitochondrial genes: *Cytb* (1,140 base pairs [bp]), 12S rRNA (*12S*; 869 bp), and cytochrome oxidase I (*COI*; 1,545 bp). Five nuclear genes were also sequenced, including the 5’ end of exon 1 of the single-copy interphotoreceptor retinoid binding protein (*IRBP*; 1,272 bp), the growth hormone receptor gene (*GHR*; 832 bp), recombination activating protein I (*RAG1*; 1,293 bp), the mast cell growth factor protein (*MGF*; 727 bp), and 1 anonymous locus (*TBO47* from Belfiore et al. 2008; 601 bp).

DNA was extracted from approximately 25 mg of liver or kidney tissue using the DNeasy extraction kit (Qiagen, Valencia, California), following the protocol for animal tissues. DNA was amplified using the following polymerase chain reaction (PCR) conditions in a 25 µl reaction volume: 1–2 µl (50 ng) template DNA, 0.5 µl of 10 mM dNTPs (2.5 mM each of dATP, dCTP, dGTP, dTTP), 0.5 µl of primer, 2.5 µl MgCl₂ (25 mM), 1 µl 1X BSA, 2.5 µl 10X buffer, 0.1 µl *Taq* (Amplitaq Gold DNA polymerase, Applied Biosystems, Foster City, California), and sterile dH₂O. The thermal profile consisted of 95°C for 2–10 min, followed by 30–35 cycles of the following: denaturation at 95°C for 15–90 s, annealing at primer-specific temperature for 20–120 s, 1–2 min extension at 72°C, and final primer extension at 72°C for 5–10 min. PCR products were visualized on 1% sodium borate agarose gels stained with ethidium bromide or Syber Green (Zipper et al. 2004). Positive amplicons were then purified with a 20% polyethylene glycol clean-up solution or an exonuclease I and shrimp alkaline phosphatase solution (ExoSAP-IT; Affymetrix Inc., Santa Clara, California). A list of primer sequences and their annealing temperatures can be found in Appendix 2.2.

Both DNA strands were sequenced from clean reaction products using 1.5–2.1 µl of 5X sequencing buffer (Applied Biosystems), 1 µl of 10 mM primer, 1–1.5 µl template, 0.35–0.5 µl Big Dye Terminator cycle-sequencing kit 3.1 (Applied Biosystems), and 1.5–2.1 µl of sterile
Cycle sequencing conditions consisted of 95°C for 5 min, followed by 40 cycles of the following: denaturation at 95°C for 30 s, annealing at 50°C for 10 s, and annealing at 60°C for 4 min. Cycle sequencing product was cleaned using Sephadex G-50 (GE Healthcare, Piscataway, New Jersey) in 400 µl DTR 96-well plates (Phenix Research Products, Candler, North Carolina). Amplicons were separated and visualized on an Applied Biosystems 3100 Genetic Analyzer housed in the LSU Museum of Natural Science. Sequences were assembled and edited in Sequencher 4.7 (Gene Codes Corp., Ann Arbor, Michigan) and Geneious 5.2 (Drummond et al. 2011). Alignments were generated using the MUSCLE algorithm in Geneious and checked by eye. The 12S alignment was explored in the program GBlocks (Castresana 2000) and uninformative gaps and indels were removed.

Phylogenetic analyses.—Bayesian Inference (BI) analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and maximum likelihood (ML) analyses were implemented in RaxML 7.3.0 (Stamatakis 2006) via the CIPRES Gateway (Miller et al. 2010). We evaluated the most appropriate models for each gene in MrModelTest 2.4 (Nylander 2004), which provides models appropriate for both BI and ML analyses. We selected the best model using the Akaike Information Criterion (Posada and Buckley 2004). The GTR+I+G model was selected for *Cytb*, 12S, and *COI*, the HKY+I model was selected for *IRBP*, *GHR*, *MGF*, and *Rag1*, and the HKY model was selected for *TBO47*.

In both sets of phylogenetic analyses (BI and ML), sequences were concatenated and then partitioned by gene, allowing for each gene to be analyzed using its appropriate evolutionary model. In the BI analysis, model parameters were treated as unknown variables with uniform priors. Two independent runs were initiated with random starting trees, an initial melting point of 0.25, and run for at least 9 x 10⁶ generations with 4 incrementally heated chains (Metropolis-
coupled Markov chain Monte Carlo [MCMC]; Huelsenbeck and Ronquist 2001) sampled every 100 generations. Convergence and stationarity were assessed using Tracer v1.5 (Rambaut and Drummond 2007). Trees generated before stationarity of log-likelihood scores was reached were discarded. Clade support was assessed using Bayesian posterior probabilities (pp). ML gene-partitioned analyses were run for 1,000 bootstraps (bs), using the GTRCAT model for the bootstrapping phase in RaxML and GTRGAMMA model for the tree inference phase. Sequences are deposited in GenBank (Appendix 4.2).

Divergence dating.—Estimates of divergence dates based on molecular analyses exist for Thomomys (Belfiore et al. 2008; Spradling et al. 2004), but those studies included only representatives of the T. umbrinus complex with 2n = 76 (now recognized as T. atrovarius and T. sheldoni) and not 2n = 78 (T. umbrinus). Divergence dates were estimated in BEAST 1.7.4 (Drummond and Rambaut 2007) using one representative each of T. atrovarius, T. sheldoni, and T. nayarensis, 1 representative of each of the 3 clades within T. umbrinus, 1 individual of T. bottae (for complete sampling of Mexican Thomomys), plus T. talpoides (representing the subgenus Thomomys) and O. hispidus as outgroups. The first BEAST analysis included all 8 genes and used a linked tree topology; the second included only Cytb, as the inclusion of multiple loci often does not measurably increase accuracy in divergence dating (Edwards and Beerli 2000). MrModelTest was used to select the appropriate evolutionary model for each gene. An uncorrelated, lognormal, relaxed clock was used, and the trees were estimated under the Yule prior from a randomly generated starting tree.

For divergence analyses, a lognormal prior was placed on the tree root with an initial value of 0.2, mean of 2.25, standard deviation of 0.075 and an offset of 4.5, allowing an error range of 6.4 to 7.1 mya (the estimated minimum divergence date of the tribes Thomomyini and
Geomyini based on fossil information [Tedford et al. 2004]). The analysis was run for $10^7$
generations and sampled every $50^2$ generations. Convergence of the MCMC was assessed using
Tracer v1.5, where high effective sample sizes (ESS > 500) for all parameters were confirmed,
and at least 2 runs were completed to confirm convergence. Runs were combined using
LogCombiner, and after discarding a 10% burn-in, a maximum clade credibility tree was
generated in TreeAnnotator (BEAST 1.7.2 package; Drummond and Rambaut 2007).

Species tree analyses.—To assist in making informed decisions about subspecies
designations within *T. umbrinus*, species tree analyses were run in *BEAST 1.7.4* (Heled and
Drummond 2010). This program within the BEAST package co-estimates multiple gene trees
within a shared species tree in a coalescent framework using a Bayesian MCMC algorithm. The
same *T. umbrinus* taxa and loci used in the BI and ML analyses were used in this analysis, along
with 2 representatives each of *T. sheldoni* and *T. atrovarius* (representing the 2 subspecies
recognized within each of those species). Specimens representing 14 of the 18 currently
recognized *T. umbrinus* subspecies were included in the analysis. Genetic samples were not
available for *T. u. atrodorsalis*, *T. u. newmani*, and *T. u. supernus*, and only Cytb sequences were
available for *T. u. camargensis*. Individuals were coded as subspecies *a priori* based on
published records.

Because the BI and ML analyses showed many of the currently recognized *T. umbrinus*
subspecies to be paraphyletic, taxa were coded in the species tree analyses based on membership
in genetically defined lineages, rather than subspecies. The lineages used in the Central Plateau
group were coded as “*juntae*” (localities 9–12 and 16–20; subspecies *juntae* and *nelsoni*) and
“goldmani” (localities 15 and 21–24; subspecies *nelsoni* and *goldmani*). The lineages used in
TMVB group were coded as “*durangi*” (localities 28–30; all *T. u. durangi*), “*zacatecae*”
(localities 31, 35, and 38; subspecies durangi, sheldoni, and zacatecae), “supernus” (localities 36, 39–41; subspecies zacatecae, arriagensis, potosinus, and pullus), and “umbrinus” (localities 42–46; all T. u. umbrinus). The Northern Desert individuals were coded based on their subspecies because this clade did not show any strong paraphyly. The same models, clocks, sampling, and convergence estimation used in the divergence dating were used in these analyses.

**Allozyme analyses.**—Allozyme data originally published by Hafner et al. (1987) were reanalyzed to further investigate relationships within T. umbrinus. Genotype assignment tests for 22 polymorphic loci sampled from 17 populations (N = 284 individuals) were performed in Arlequin 3.1 (Excoffier et al. 2005). These tests compute the log-likelihood of the genotypes of the individuals in each clade under the assumption that they were taken from the same population and have equal allele frequencies (Paetkau et al. 1997; Waser and Strobeck 1998). The output from this test allows us to infer population membership of each genotype.

**Morphometric analyses.**—Twelve cranial characters were measured on 211 individuals to the nearest 0.1 mm using hand-held digital calipers: cranial width (CW), diastema length (DIA), width of interorbital constriction (IOC), mastoid breadth (MB), length of maxillary tooth row (MTR), nasal length (NL), occipital-nasal length (ONL), occipital-incisor length (OIL), rostral width (RW), zygomatic breadth (ZB), breadth of mandible (BM), and mandible length (ML). Adult female specimens were used in the morphometric analyses because of the extreme sexual dimorphism in pocket gophers (Hafner et al. 2004; Patton and Smith 1990; Smith and Patton 1988). Specimens were judged to be adult based on fusion of the exoccipital-supraoccipital and basioccipital-basispheniod sutures (Daly and Patton 1986).

Morphometric analyses were performed on the 3 genetic clades (Central Plateau, Northern Desert, and TMVB), the currently recognized subspecies, and the monophyletic genetic
lineages identified in the species tree analyses. Statistical analyses of the morphometric data were conducted using SPSS 19 (IBM, Armonk, New York). Data were assessed for normality and examined for extreme outliers, which were removed from further analyses. Data were transformed ($\bar{X} = 0$, $SD = 1$) and a multivariate analysis of variance (MANOVA) was used to test the null hypothesis of no significant difference between a priori groups. A post hoc analysis of the MANOVA was assessed with Tukey’s HSD. A principal components analysis (PCA) was performed using a varimax rotation to reduce the 12 variables and explore the dimensionality of the data. Direct discriminant function analysis (DFA) was performed to generate discriminant functions to predict group membership and evaluate if individuals could be properly assigned to their a priori groups.

4.3 RESULTS

Phylogenetic analyses.—Inspection of the BI and ML phylogenetic trees generated from the concatenated, gene-partitioned sequence data revealed strong support for monophyly of the *T. umbrinus* species complex, as well as monophyly of *T. umbrinus* and each of the 3 genetic clades (Central Plateau, Northern Desert, and TMVB) within *T. umbrinus* (Fig. 4.2). Support for the sister relationship between the Central Plateau and Northern Desert clades was high in the BI analysis ($pp = 0.95$) but only moderate in the ML analysis ($bs = 80$). As seen in previous studies of the *T. umbrinus* complex (Hafner et al. 2011, Chapter 2 in this dissertation), relationships among *T. sheldoni*, *T. nayarensis*, and *T. atrovarius* are unresolved (Fig. 4.2), which may be the result of a rapid phyletic radiation in this clade (Spradling et al. 2004).

Genetic breaks within *T. umbrinus* did not correspond well with traditional subspecies boundaries. Of the 8 subspecies represented by specimens from more than a single locality, only 1 (*T. u. umbrinus*) was monophyletic. The 4 subspecies in the Northern Desert clade showed
Fig. 4.2.—Genetic relationships among species of *Thomomys*, with emphasis on *T. umbrinus*, based on gene-partitioned Bayesian and maximum likelihood analyses of 3 mitochondrial and 5 nuclear genes (8,279 bp). The maximum likelihood topology is shown. Black circles indicate well-supported nodes, grey circles indicate nodes with high posterior probabilities but weaker bootstrap support, and nodes with weak support (posterior probability < 0.95 and bootstrap support < 80%) are collapsed. Numbers at the tips of branches refer to localities mapped in Fig. 4.1 and listed in Appendix 4.1. Currently recognized subspecies epithets follow locality numbers. Diploid numbers are indicated on major branches of the tree.

Little genetic structuring beyond support for a western (*intermedius* + *sonoriensis*) clade (Figs. 4.1 and 4.2). The Central Plateau group was divided into a northern clade containing the subspecies *juntae*, *nelsoni*, and *camargensis* and a southern clade containing 4 populations of *T. u. goldmani* and 1 of *T. u. nelsoni* (locality 15; Fig. 4.2). Placement of the individual from locality 15 in the southern clade is problematic considering that it is geographically proximate to 5 other *T. u. nelsoni* populations placed in the northern clade (Fig. 4.1). The TMVB group was divided into 3 clades, including a highly divergent northern clade comprised of 3 populations of...
T. u. durangi, a central clade containing 1 population each of T. u. durangi, T. u. zacatecae, and T. u. sheldoni, and a southern clade containing 1 population each of the subspecies arriagensis, potosinus, zacatecae, and pullus, and all 5 populations of T. u. umbrinus.

**Species tree analyses.**—Analyses revealed 4 well-supported clades (boxes A–D; Fig. 4.3) within T. umbrinus that are phylogenetically concordant with results of the BI and ML analyses (Fig. 4.2) except for placement of clade C. Northern Desert and Central Plateau subspecies showed little genetic structure. Visual inspection of the posterior distribution of species trees revealed little support for inclusion of clade C (T. u. durangi) in the TMVB group, as shown in Fig. 4.2; instead, this lineage showed a weak relationship to the Central Plateau group. In the species tree analysis, the individual gene trees for 3 nuclear genes (IRBP, MGF, and GHR) supported the Central Plateau affinity for clade C, whereas the 3 mitochondrial genes supported the TMVB relationship. Relationships within clades A, B, and D were only partially resolved (Fig. 4.3).

**Divergence dating.**—Divergence dates estimated in this analysis (Table 4.1) were older than those previously reported by Belfiore et al. (2008), with the exception of the split between subgenera Thomomys and Megascapheus; the multi-locus analysis was equal to the previously published mean. The analysis based on the multi-locus dataset yielded dates that were on average 7-22% younger than dates estimated using Cytb only, but the highest posterior density intervals overlapped for all splits (Table 4.1). The tree generated in the multi-locus BEAST analysis (not shown) showed a weakly supported sister relationship between the Central Plateau and TMVB groups, whereas the Cytb BEAST analysis agreed with the BI and ML analyses (Fig. 4.2) in showing a weakly supported sister relationship between the Northern Desert and Central Plateau clades.
Fig. 4.3.—Phylogram of the consensus of species trees in the *Thomomys umbrinus* complex. Northern Desert, Central Plateau, and Trans-Mexico Volcanic Belt (TMVB) refer to distinct genetic clades within *T. umbrinus*. Published subspecies epithets for *T. umbrinus* are listed at the tips. The *zacatecae* and *supernus* lineages listed within TMVB represent monophyletic clades comprised of multiple subspecies, as indicated by prior exploration of gene trees and concatenated data. Suggested new lineages possibly diagnostic of new subspecies (*intermedius*, *goldmani*, *durangi*, and *umbrinus*) are designated. Black circles indicate strong posterior probability support > 0.95 and branches that did not meet that level of support were collapsed.

Genetic differentiation.—The 3 clades within *T. umbrinus* showed high levels of pairwise genetic differentiation at the *Cytb* locus, ranging from 10% (between Northern Desert and Central Plateau) to 21% (between Central Plateau and TMVB). Although genetic differentiation within the Northern Desert and Central Plateau groups was fairly low (up to 6.6% in the Central Plateau group), the TMVB group showed an average of 15.8% within-group differentiation. The *T. u. durangi* lineage in the concatenated and species tree analyses averaged 15.3% divergence from the *umbrinus* lineage.
Table 4.1.—Mean estimated divergence dates (with highest posterior density intervals in parentheses) for select groups of *Thomomys* generated in a multi-locus BEAST analysis (Drummond and Rambaut 2007) of 3 mitochondrial and 5 nuclear genes and a separate analysis including only cytochrome *b* (*Cytb*). Divergence estimates from Belfiore et al. (2008) are provided for comparison. Divergence dates are listed for: 1) divergence of subgenus *Thomomys* from subgenus *Megascapheus*; 2) divergence of the *T. umbrinus* complex (*T. umbrinus*, *T. atrovarius*, *T. sheldoni*, and *T. nayarensis*) from *T. bottae*; 3) divergence of *T. umbrinus* from other members of the *T. umbrinus* species complex; 4) divergence of *T. atrovarius* from *T. sheldoni* and *T. nayarensis*; and 5) divergence of the 3 clades within *T. umbrinus* (Northern Desert, Central Plateau, and Trans-Mexico Volcanic Belt [TMVB]). For the multi-locus analysis, this is the split of Central Plateau from the other 2 clades; for the *Cytb* only analysis, it is the split of TMVB from the other 2 clades.

<table>
<thead>
<tr>
<th>Divergence</th>
<th>Multi-locus</th>
<th><em>Cytb</em> only</th>
<th>Belfiore et al. (2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Thomomys</em>/<em>Megascapheus</em></td>
<td>5.93 (5.02 – 6.82)</td>
<td>6.39 (5.67 – 7.01)</td>
<td>5.93 (2.5 – 9.6)</td>
</tr>
<tr>
<td>2. <em>T. bottae</em>/<em>T. umbrinus</em></td>
<td>3.45 (2.79 – 4.16)</td>
<td>4.04 (3.29 – 4.80)</td>
<td>1.93 (0.2 – 1.3)</td>
</tr>
<tr>
<td>3. <em>T. umbrinus</em></td>
<td>3.01 (2.39 – 3.61)</td>
<td>3.60 (2.93 – 4.30)</td>
<td>—</td>
</tr>
<tr>
<td>4. <em>T. atrovarius</em></td>
<td>2.66 (2.11 – 3.19)</td>
<td>3.21 (2.52 – 3.86)</td>
<td>0.49 (0.15 – 0.88)</td>
</tr>
<tr>
<td>5. within <em>T. umbrinus</em></td>
<td>1.99 (1.49 – 2.46)</td>
<td>2.56 (1.96 – 3.18)</td>
<td>—</td>
</tr>
</tbody>
</table>

Allozyme analyses.—The genotype assignment tests using allozyme data from Hafner et al. (1987) showed a general north-to-south cline in log-likelihood scores (Fig. 4.4). Most disruptions in the cline occur in regions that were not sampled for this study (e.g., between the Ventura and Patzcuaro localities) and appear to be sampling artifacts. However, the disruption between the Morcillo and Sombrerete samples (localities 29 and 30 in Fig. 4.1) occurs over a relatively short distance (120 km) with no obvious physiographic barriers to gene flow.

Morphometric analyses.—Two variables (CW and MB) departed significantly from normality based on a Kolmogorov-Smirnov test with a Lilliefors correction (*P* < 0.05). These variables were removed from the MANOVA but were included in the PCA and DFA since these analyses are robust to deviations from normality not caused by outliers (Tabachnick and Fidell 1996). The PCA of transformed data resulted in 2 factors with eigenvalues exceeding 1.0 that together accounted for 73.1% of variation in the data set. An examination of a scatter plot of PC
Fig. 4.4.—Average log-likelihood scores with 95% confidence intervals from genotype assignment tests for 22 polymorphic allozyme loci for specimens from the Northern Desert, Central Plateau, and Trans-Mexico Volcanic Belt clades of *T. umbrinus*. Populations are ordered in a north-to-south direction and plotted by latitude. The right inset box lists the general locality names followed by sample size (*n*). Numbers preceding locality names and listed to the left of the circles match those in Fig. 4.1. Assignment scores are calculated as though the Northern Desert clade were the source population.

1 and PC 2 scores was not informative for identification of any of the genetic groups or subspecies (data not shown).

A 1-way MANOVA performed on the untransformed morphometric data for the 3 genetically defined clades (Northern Desert, Central Plateau, and TMVB; Fig. 4.2) was significant for all 3 groups (Wilks’ *λ* = 0.34, *F*<sub>24,372</sub> = 11.17; Pillai’s Trace = 0.76, *F*<sub>24,374</sub> = 9.57; Hotelling’s Trace = 1.67, *F*<sub>24,370</sub> = 12.85; *P* < 0.0001 for all 3 tests). Exploring Tukey’s HSD post
hoc tests on the MANOVA revealed that, on average, members of the Central Plateau clade were larger than those of the other 2 clades at every measurement except RW and IOC (where Northern Desert had the largest measurements), while the Northern Desert clade was smallest at every measurement except ONL, NL, RW, and IOC. TMVB was always intermediate in size, except for ONL and NL, which were smaller than corresponding measurements in the other 2 clades. Specimens from the Central Plateau clade were significantly larger than those of the Northern Desert clade for every measurement except ONL, RW, and IOC, and Central Plateau specimens were significantly larger than those of the TMVB clade for ONL and NL ($P < 0.05$).

The direct DFA generated 2 significant canonical discriminant functions explaining 100% of the total variance. ONL had a strong negative loading (-2.18) on DF 1 and a strong positive loading on DF 2 (1.14). OIL had a strong negative loading (-1.43) on DF 2. Examination of the first 2 discriminant functions showed broad overlap between the 3 clades (Fig. 4.5a). Overall, 81.7% of the individuals were classified correctly into their a priori groups: 47.1% for Central Plateau; 87.7% for Northern Desert; 89.6% for TMVB specimens.

In the morphometric analysis of the 18 currently recognized subspecies, sample sizes ranged from 2 to 45 individuals per subspecies. Six significant discriminant functions accounted for 91.7% of the total variation and only 72.6% of individuals were correctly classified into their correct subspecies. Only 6 subspecies had more than 80% individuals correctly classified: *T. u. emotus* (80%), *T. u. intermedius* (91.3%), *T. u. juntae* (91.7%), *T. u. potosinus* (85.7%), *T. u. sheldoni* (85.7) and *T. u. umbrinus* (86.7%).

The morphometric analysis of the 4 genetically defined lineages within the TMVB clade (Figs. 4.2 and 4.3) generated 2 significant discriminant functions that accounted for 79.6% of the total variation (Fig. 4.5b). ONL had a high, negative loading on DF 1 (-1.9) and a high, positive
Fig. 4.5.—Distribution of discriminant function scores for 134 specimens of *Thomomys* on the first 2 discriminant functions (DF1 and DF2) based on 12 cranial measurements. a) Comparison of specimens from the Northern Desert, Central Plateau, and Trans-Mexico Volcanic Belt clades of *T. umbrinus*. b) Comparison of specimens representing 4 genetically distinct lineages within the TMVB clade, as identified in Figs. 4.2 and 4.3: *durangi*, *zacatecae*, *supernus* and *umbrinus*. Ellipses enclose ≥ 80% of points to illustrate degree of overlap among groups.

loading on DF 2 (1.18). OIL had a high, positive loading on DF 1 (2.39) and NL had a high, negative loading on DF 2 (-1.03). Correct classification of individuals was 77.4% overall, with 90% correct classification for the *zacatecae* group, 84.4% for the *umbrinus* group, 72.7% for the *durangi* group, and 67.5% for the *supernus* group.

Because the *zacatecae*, *supernus*, and *umbrinus* groups formed an unresolved trichotomy in the species tree analyses (Fig. 4.3), we combined them into a single group and compared this new group to the *durangi* group. The percentage of correctly classified *durangi* individuals remained as before (72.7%), but 97.9% of individuals were correctly classified into the new *umbrinus* lineage.
4.4 DISCUSSION

Genetic divergence in the *Cytb* gene in mammals is frequently used as a general indicator of species status when reproductive or genetic isolation between populations cannot be tested directly in the field (Bradley and Baker 2001). Yet relying on *Cytb* divergence to infer species status is problematic when studying species, such as pocket gophers, that have unusually high rates of *Cytb* sequence evolution (Spradling et al. 2001) and where conspecific populations often show levels of *Cytb* divergence equal to or greater than that measured between other well-defined species of mammals (Hafner et al. 1983; Patton and Yang 1977). Multi-locus genetic analyses coupled with the advent of species tree analyses may help resolve taxonomic issues in organisms with unusually high rates of *Cytb* evolution. However, taxa that have undergone recent and rapid radiations, such as the Geomyidae, may present an even greater challenge to taxonomists because of short internodal branches that are difficult to resolve and the potentially confounding effects of incomplete lineage sorting of haplotypes.

The multi-locus analyses (Fig. 4.2) and species tree analyses (Fig. 4.3) confirm the monophyly of all *T. umbrinus* populations with a diploid number of 78. Within *T. umbrinus*, most evidence supports a sister relationship between the Northern Desert and Central Plateau clades. Whereas allozyme data presented in Chapter 2 showed a sharp discordance in genotype assignment scores between individuals of *T. umbrinus* and *T. sheldoni*, the three *T. umbrinus* clades show a generally smooth cline in genetic assignment scores (Fig. 4.4) and most disruptions in the cline appear to result from gaps in sampling.

The 3 genetically defined clades that comprise what we now recognize as *T. umbrinus* show levels of *Cytb* differentiation ranging from 10% to 21%. Based on this evidence alone, it might seem reasonable to classify them as 3 separate species. However, considerable evidence
suggests that these clades, although potentially incipient species, are not genetically isolated, which is our principal criterion for species status. These clades share a derived diploid number of 2n = 78, which means that interbreeding between the clades is unlikely to result in meiotic breakdown caused by mating between pocket gophers with different diploid numbers (Patton and Dingman 1968). The 3 clades show a generally smooth cline in genotype assignments (Fig. 4.4), share nuclear haplotypes at 5 of 6 loci examined, and their genetic distances follow an isolation-by-distance pattern (see Chapter 2); all suggestive of current or recent gene flow. In contrast, the recently resurrected species *T. sheldoni* (Chapter 2) shared no haplotypes with *T. umbrinus* at the seven loci examined, had a different diploid number (2n = 76), and despite having populations within two km or less of *T. umbrinus* populations, showed no evidence of gene flow. Whereas considerable evidence points to genetic isolation of *T. sheldoni* from other Mexican pocket gophers, we have no evidence of genetic isolation among the 3 clades within *T. umbrinus*.

Understanding the phylogeography of *T. umbrinus* is difficult given the apparent recent and rapid radiation of the lineage (Spradling et al. 2004). Current fossil evidence suggests origin of the genus *Thomomys* in the western United States during late Miocene or early Pliocene with subsequent radiation and expansion of the subgenus *Megascapheus* into the southwestern United States and Mexico (Mooser and Dalquest 1975; Paleobiology Database 2013). Today the 4 species of the *T. umbrinus* species complex are almost exclusively Mexican, and Mexico appears to be the center of diversification of the complex.

Divergence estimates suggest that the common ancestor of the *T. umbrinus* complex diverged from *T. bottae* stock sometime between 3.4 and 4 mya, an earlier estimate than previously published in Belfiore et al. (2008). *T. atrovarius* appears to have been the first lineage to diverge from *T. bottae* (see Chapter 3), although this relationship lacks strong statistical
support in the ML analysis (pp = 1, but bs = 50). If *T. atrovarius* is basal within the *T. umbrinus* species complex, then it is likely that its divergence from *T. bottae* took place somewhere along the Pacific coast of present day Sinaloa (location marked A in Fig. 4.6) where the habitat today shows a rather dramatic shift from Sonoran desert scrub (occupied by *T. bottae*) to thornscrub forest (occupied by *T. atrovarius*). The pattern of diversification within *T. atrovarius* (Hafner et al. 2011) suggests a northern origin and southward expansion of the species, with older lineages distributed in the north and more recently evolved lineages in the south.

The divergence of *T. sheldoni*, *T. nayarensis*, and *T. umbrinus* from presumed ancestral *T. atrovarius* stock occurred too rapidly to resolve with current molecular data (Fig. 4.2 and 4.3; see also Chapter 2). The current southern distribution of *T. nayarensis* and presence of *T. nayarensis*, *T. sheldoni*, and *T. atrovarius* in close proximity in this region (all within a circle with radius < 12 km) suggests that this major radiation within the *T. umbrinus* species complex occurred in the southern Sierra Madre Occidental near present day Nayarit (B, Fig. 4.6).

From their presumed site of origin in northeastern Nayarit, *T. sheldoni* spread northward in the Sierra Madre Occidental (C, Fig. 4.6) while *T. umbrinus* spread into the TMVB (D, Fig. 4.6) and northward into the Central Plateau. Eventually, *T. umbrinus* (2n = 78) came into secondary contact with *T. sheldoni* (2n = 76) in northwestern Chihuahua (E, Fig. 4.6) and *T. bottae* (2n = 76) in the southwestern United States (F, Fig. 4.6) where meiotic imbalances caused by diploid number differences prevented genetic introgression at contact zones.

Unlike other members of the *T. umbrinus* species complex, *T. umbrinus* populations are found in a remarkable range of habitats. Whereas populations of *T. atrovarius* are restricted to dry, thornscrub forests (Hafner et al. 2011) and *T. sheldoni* populations occur almost exclusively in pine forest habitats above 2,000 m (Chapter 2), *T. umbrinus* populations have been

69
documented at elevations exceeding 4,000 m and in habitats ranging from dry, desert scrublands to pine forests near timberline. *T. nayarrensis* is known only from 2 localities to date: 1 in pine forest habitat and the other in a human-disturbed ecotone between pine forest and thornscrub forest habitats (Chapter 3).

The 3 major clades of *T. umbrinus* have large (>120 km) gaps between their distributions (Fig. 4.6), with no known capture records from the intervening areas. These gaps consist primarily of rocky, barren habitat seemingly unsuitable for permanent colonization by pocket gophers, but it is likely that isolated populations exist in the gaps, thereby facilitating the gene flow we detected in this study.

Morphology-based taxonomy is problematic in pocket gophers because of their extreme morphological conservatism coupled with environmentally induced variation in body size and pelage quality and coloration (Hafner et al. 2008; Hafner et al. 2004; Patton and Brylski 1987; Smith and Patton 1988). Overreliance on body size and pelage characteristics to define taxonomic groups is what led, in part, to the overabundance of named subspecies in Hall (1981; 229 subspecies in *T. bottae* + *T. umbrinus*). Although morphological data may be of dubious value for discriminating species and subspecies of pocket gophers, it often provides evidence in support of taxonomic decisions, especially when it is concordant with genetic breaks.

Here we follow the recommendations of Lidicker (1962) and recognize only diagnosable and genetically monophyletic groups as subspecies. The currently recognized subspecies in the Northern Desert and Central Plateau clades (Fig. 4.1) are not monophyletic (Fig. 4.2), and the divergence event that divides the Central Plateau clade into two units is not reflected in the morphometric analysis. Accordingly, we recognize only one subspecies per clade, with *T. u.*
Fig. 4.6.—Scenario of possible diversification and expansion of *Thomomys* in Mexico, represented by dotted arrows and letters: A, divergence of *T. atrovarius* from *T. bottae* in Sinaloa; B, radiation of *T. umbrinus*, *T. sheldoni* and *T. nayarensis* from *T. atrovarius* in Nayarit; C, northward expansion of *T. sheldoni* through the Sierra Madre Occidental; D, expansion of *T. umbrinus* south through the Trans-Mexico Volcanic Belt and north through the Central Plateau; E, secondary contact between *T. umbrinus* and *T. sheldoni* in northeastern Chihuahua; F, secondary contact between *T. umbrinus* and *T. bottae* in the southwestern United States. Horizontal lines in *T. atrovarius*, *T. sheldoni*, and *T. umbrinus* distributions indicate general borders between newly revised subspecies. The location of the Pleistocene-era pluvial Lake Xalisco (De Cserna and Alvarez 1995) is shown. This lake likely had an influence on expansion of *Thomomys*.
*intermedius* representing the Northern Desert clade and *T. u. goldmani* representing the Central Plateau clade (Fig. 4.6).

The TMVB clade shows high levels of within-clade genetic differentiation, reaching almost 16% divergence compared to a maximum of 6% divergence within the other two clades. Three of the TMVB clades (*zacatecae*, *supernus*, and *umbrinus*) showed a high degree of morphological overlap, but separated fairly well from the *durangi* lineage in multivariate space.

Species tree analyses of the TMVB group were generally concordant with results of the BI and ML analyses, albeit with weaker statistical support. Relying on a concatenation of a multi-locus dataset to make taxonomic decisions may be undesirable, as the independent gene trees may be incongruent with the true species tree (Degnan and Rosenberg 2006; Kubatko and Degnan 2007). However, relying on species tree analyses requires confidence in *a priori* assignments, which is difficult to gauge when studying pocket gopher subspecies that were named long ago without the aid of modern genetic tools. Where questionable assignments exist, reassigning or removing the problematic individual(s) may be the best option (Leaché 2009).

In this case we relied upon prior information gathered from the individual gene trees and the gene-partitioned, concatenated topology to assign taxa to monophyletic genetic groups rather than rely on traditional subspecies designations. This resulted in the species tree analyses agreeing with the ML/BI analyses in most respects, aside from the placement of the *T. u. durangi* lineage. However, the lack of statistical support for the alternative placement of this lineage in the species tree did not alter our final conclusions after unsupported branches were collapsed.

For the purposes of diagnosing genetic units representative of subspecies, we choose to be guided by the species tree analysis. Although the *zacatecae*, *supernus*, and *umbrinus* clades had relatively strong support in the concatenated analyses, the lack of support in the species tree
analyses, relatively low pair-wise genetic divergence values at Cyth seen among these 3 lineages, and the fairly broad overlap in morphometric space lead us to propose combining the zacatecae, supernus and umbrinus lineages into one subspecies. We recommend 2 subspecies within the TMVB clade: T. u. durangi in southern Durango and extreme northwestern Zacatecas, and T. u. umbrinus, comprised of the remaining subspecies from southeastern Zacatecas through the TMVB region of central Mexico. A synonymy of T. umbrinus and the newly recognized subspecies follows.

_Thomomys_ Wied-Neuwied, 1839

Smooth-toothed Pocket Gophers

_Diplostoma_ Richardson, 1829:206. Type species _D. bulbivorum_ Richardson, 1829:206.


_Thomomys_ Wied-Neuwied, 1839:377. Type species _T. rufescens_ Wied-Neuwied, 1839:378 (= _T. talpoides rufescens_).


_Megascapheus_ Elliot, 1903:190. Type species _Diplostoma bulbivorum_ Richardson, 1829:206.


**Comments.**—The 9 recent species of _Thomomys_ (Patton 2005) are allocated into 2 subgenera, _Thomomys_ and _Megascapheus_ (Thaeler 1980). Mexican species of _Thomomys_, currently _T. bottae, T. umbrinus_, and the recently resurrected _T. atrovarius_ (Hafner et al. 2011) and _T. sheldoni_ (Chapter 2) are members of the subgenus _Megascapheus_. A synonymy of _T. umbrinus_ with diagnosis of four subspecies follows.
Thomomys umbrinus intermedius Mearns, 1897

*Thomomys fulvus intermedius* Mearns, 1897:719. Type locality: “Summit of Huachuca Mountains, Arizona (altitude 9,000 feet).”

*T. burti* Huey, 1932:158. Type locality “Madre Canyon, Santa Rita Mountains, Arizona (altitude 6,000 feet).”

*T. f. emotus* Goldman, 1933:76. Type locality: “Animas Peak, Animas Mountains, New Mexico (altitude 8,000 feet).”

*T. burti quercinus* Burt and Campbell, 1934:150. Type locality: “Peña Blanca Spring, Pajarito Mountains, Arizona (near Mexican boundary, north of monument 128).”


*T. u. madrensis* Nelson and Goldman, 1934:115. Type locality: “Pilares Canyon, 10 miles northeast of Colonia Garcia, and about 25 miles southwest of Casas Grandes, Chihuahua, Mexico (altitude 6,400 feet).”

*T. u. caliginosus* Nelson and Goldman, 1934:116. Type locality: “Altamirano, Sierra Madre, northwestern Chihuahua, Mexico (altitude 8,000 feet), near Sonora boundary west of Casas Grandes.”


*T. u. sonoriensis* Nelson and Goldman, 1934:118. Type locality: “10 miles east of Chinapa, Sonora River Valley, northern Sonora, Mexico (altitude 3,000 feet).”

Geographic range.—From the Patagonia, Santa Rita, Huachuca, and Parajito mountains of southeastern Arizona and the Animas mountains of southwestern New Mexico, extending...
southward into Sonora and northwestern Chihuahua, terminating approximately 2 km south of Colonia Garcia, Chihuahua (approximately 29.95° N).

Comments.—Lange (1959) placed *T. u. burti* and select individuals from *T. bottae proximus* (from Canelo Gate and 1 mi. N of Fort Huachuca) under synonymy with *T. u. intermedius*, concluding that pocket gophers from the Santa Rita, Patagonia, and Huachuca mountains should be placed under one subspecies (*T. u. intermedius*). Likewise, Hoffmeister (1986) placed *T. u. quercinus* from the Pajarito mountains of southeast Arizona in synonymy under *T. u. intermedius*. Anderson (1972) synonymized *T. u. caliginosus* under *T. u. madrensis*. Included in *T. u. madrensis* were specimens of *T. u. chihuahuae* from Altamirano, Chihuahua and specimens of *T. u. chihuahuae* and *T. bottae divergens* from Chuhuichupa, Chihuahua (Anderson 1972). The specimens from Chuhuichupa are now recognized as *T. sheldoni chihuahuae*.

*Thomomys umbrinus goldmani* Merriam, 1901

*Thomomys goldmani* Merriam, 1901:108. Type locality: “Mapimi, Durango, Mexico (altitude 3,800 feet).”


*T. u. evexus* Nelson and Goldman, 1934:115. Type locality: “Mount San Gabriel, northwestern Durango, Mexico (between 7,000 and 8,000 feet altitude).”


*T. u. camargensis* Anderson, 1972:288. Type locality: “1 mi. NW Camargo, Chihuahua (3,950 ft)”

*T. u. juntae* Anderson, 1972:292. Name combination

**Geographic range.**—Central Chihuahua extending southward into central eastern Durango and extreme southwestern Coahuila.

**Comments.**—Anderson (1972) placed *T. u. evexus* as a junior synonym of *T. u. nelsoni*.

*Thomomys umbrinus durangi*, Nelson and Goldman, 1934

*T. u. durangi* Nelson and Goldman, 1934:114. Type locality: “Durango, Durango, Mexico.”

**Geographic range.**—Restricted to southwestern Durango and extreme northwestern Zacatecas.

*Thomomys umbrinus umbrinus*, Richardson, 1829

*Geomys umbrinus* Richardson, 1829:202. Type locality: “Cadadaguis, a town in southwestern Louisiana” which cannot be identified.

*Thomomys umbrinus* Bailey, 1906:3. Name restricted to vicinity of Boca del Monte, Veracruz, Mexico, but probably Puebla, Mexico.

*T. u. umbrinus* Bailey, 1915:89. Name combination.

*T. orizabae* Merriam, 1893:145. Type locality: “Mt. Orizaba, Puebla, Mexico (altitude, about 9,500 feet).”


*T. u. albicularis* Nelson and Goldman, 1934:106. Type locality: “El Chico, Sierra de Pachuca, Hidalgo, Mexico (altitude 9,000 feet).”

*T. peregrinus* Merriam, 1893:146. Type locality: “Salazar, México, Mexico (altitude 10,300 feet).”

T. u. martinensis Nelson and Goldman, 1934:108. Type locality: “San Martin Texmelucan, Puebla, Mexico (altitude 7,400 feet).”

T. u. tolucae Nelson and Goldman, 1934:109. Type locality: “Volcano of Toluca, México, Mexico (north slope, altitude 9,500 feet).”

T. u. vulcanius Nelson and Goldman, 1934:109. Type locality: “Volcano of Popocatepetl, México, Mexico (altitude 12,900 feet).”

T. u. supernus Nelson and Goldman, 1934:110. Type locality: “Santa Rosa, about 7 miles northeast of Guanajuato, Guanajuato, Mexico (altitude between 9,500 and 10,000 feet).”

T. u. potosinus Nelson and Goldman, 1934:111. Type locality: “La Tinaja, about 20 miles northeast of San Luis Potosí, Mexico (altitude 6,000 feet).”

T. u. atrodorsalis Nelson and Goldman, 1934:111. Type locality: “Alvarez, San Luis Potosí, Mexico (altitude 8,000 feet).”

T. u. zacatecae Nelson and Goldman, 1934:112. Type locality: “Berriozabel, Zacatecas (altitude 6,000 feet).”

T. u. enixus Nelson and Goldman, 1934:112. Type locality: “Sierra Moroni, near Plateado, Zacatecas, Mexico (altitude 8,500 feet).”

T. u. pullus Hall and Villa, 1948:251. Type locality: “5 mi. S Pátzcuaro, Michoacán, Mexico (altitude 7,800 feet).”

T. u. newmani Dalquest, 1951:361. Type locality: “7 km northwest of La Palma (village 12 km northwest of Salinas), San Luis Potosí, Mexico.”

T. u. arriagensis Dalquest, 1951:361. Type locality: “1 km south of Arriaga, San Luis Potosí, Mexico.”
Geographic range.—Distributed from east-central Zacatecas southward through the TMVB belt into Veracruz.

Comments.—Matson & Baker (1986) placed *T. u. enixus* in synonymy under *T. u. zacatecae*. Individuals from the vicinity of Monte Escobedo, Zacatecas (locality 35, Fig. 4.1 and Fig. 4.2) were previously assigned to *T. u. sheldoni* (Matson and Baker 1986). Other individuals of this subspecies from the Sierra Madre Occidental, including the type locality, were recently elevated to species status as *T. sheldoni* (Chapter 2). Individuals from the vicinity and north of Jimenez de Teul, Zacatecas (locality 31; Fig. 4.1) were previously designated as *T. u. durangi* but should now be referred to as *T. u. umbrinus*. Castro-Campillo and Ramírez-Pulido (2000) used morphological evidence to reduce the number of subspecies of *T. umbrinus* in the TMVB from six to two (*T. u. umbrinus* and *T. pullus*). *Thomomys u. atrodorsalis* and *T. u. newmani* in San Luis Potosí and *T. u. supernus* in Guanajuato were not sampled genetically in this study but are found adjacent to or between other sampled subspecies, would likely not exhibit any genetic discordance, and so should be synonymized within the *T. u. umbrinus* group.
CHAPTER 5
THE ROLES OF NICHE CONSERVATISM AND COMPETITION IN A RAPID RADIATION OF FOSSORIAL MAMMALS

5.1 INTRODUCTION

Rapid radiations occur over relatively short evolutionary time scales. Many radiations are adaptive and result in new species filling a novel niche space. However, some radiations appear to be non-adaptive, where there is a rapid divergence event that has resulted in several new species that do not fill novel niche space but instead partition themselves, either in an allopatric manner or in a mosaic-type distribution (Gittenberger 1991; Rundell and Price 2009). So the absence of a species in a region not already occupied by congeners begs the question of what factors are involved in limiting species distributions (Lomolino et al. 2005; MacArthur 1972).

Random genetic processes coupled with small or patchily distributed populations or selection can result in genetically distinct units that maintain their differences upon secondary contact. Once a species becomes an independently evolving lineage and undertakes a new evolutionary trajectory, how and why it utilizes available habitats is integral to its formation and ability to colonize new areas. Many factors can interact to limit or promote species distributions into new habitats, ranging from abiotic factors, such as soil or climate, to biotic interactions including competition and parasitism (Lomolino et al. 2005).

Species that diverge in allopatry often show a high degree of niche conservatism (Pearman et al. 2008; Peterson et al. 1999), meaning that the niche remains fundamentally unchanged in sister species (Wiens and Graham 2005). Because of this, niche conservatism can act to maintain differentiation through ecological reinforcement and prevent recently evolved species from coming into secondary contact (Wiens 2004). It may be difficult to tease apart ecological niche conservatism in closely related species from conservatism inherent to their
phylogenetic relatedness, but the two causes of conservatism need not necessarily be related (Losos 2008). Ecological niche modeling and species distribution modeling can help us understand the role the ecological niche plays in species delimitation and distributions; the degree of shared climatic envelopes can address the presence or absence of a conserved niche and allow us to generate hypotheses of gene flow or dispersal limitations in keeping species separate (Wiens and Graham 2005).

Species distributions in pocket gophers (family Geomyidae) rarely overlap; instead geomyids maintain allopatric or parapatric distributions with limited interdigitation (Miller 1964). Whether this type of “contiguous allopatry” is due to competitive exclusion or different habitat requirements, or some combination of the two, is unknown. Miller (1964) found that soil tolerance and competition were critical in determining distributions of four species of pocket gophers, concluding that the species with the stricter habitat requirement were the superior competitors. However, Miller (1964) did not study closely related species of pocket gophers with similar habitat preferences, body sizes, and dispersal abilities.

Here I explore species distributions and estimate the climatic envelopes of 3 species of the Thomomys umbrinus species complex. This complex has been the subject of revision and taxonomic change in recent years (Álvarez-Castañeda 2010; Hafner et al. 2011; see also Chapters 2 and 3 in this dissertation). Until recently, the complex was considered a single species with populations having a diploid number of either 2n = 76 or 2n = 78 and up to 5 distinct genetic clades referred to as the Sierra Madre, Pacific Coast, Northern Desert, Central Plateau, and Trans Mexican Volcanic Belt clades. These clades were originally identified based on differences in chromosomes and allozymes (Hafner et al. 1987; Patton and Feder 1978) and later confirmed using multi-locus genetics (Hafner et al. 2011; Chapter 2). The Pacific Coast clade (2n
was recently elevated to species status as *T. atrovarius* (Álvarez-Castañeda 2010; Hafner et al. 2011), followed by the resurrection of *T. sheldoni* (*2n* = 76) from the Sierra Madre clade (Chapter 2). A third species, *T. nayarensis* (also *2n* = 76), was also recently described (Chapter 3). The fourth member of the clade, *T. umbrinus*, has a diploid number of *2n* = 78 and is comprised of three genetic clades (Chapter 4). A bioclimatic envelope model of *T. atrovarius* was generated by Hafner et al. (2011) and compared to some of the members of the complex. The distinctive niche occupied by *T. atrovarius* contributed to the resurrection of its species status.

Each genetic unit in this complex inhabits a discrete distribution with no documented overlap. A northern congener, *T. bottae*, has limited sympathy with *T. atrovarius* and the Northern Desert clade of *T. umbrinus* (Fig. 5.1). Patton (1973) documented limited hybridization between *T. bottae* and *T. umbrinus* and where they are sympatric in southeastern Arizona. Further investigations at this contact zone by Patton and Dingman (1968) indicated that the two congeners appear to be ecologically distinct when in sympathy, with *T. bottae* as the possible superior competitor restricting *T. umbrinus* to the less productive higher elevation habitats. In the absence of *T. bottae*, *T. umbrinus* was found at lower and intermediate elevations (Patton and Dingman 1968).

Molecular analyses show that the Geomyidae family underwent a rapid phyletic radiation during a relatively brief time in the early Blancan (ca. 5–7 mya; Lindsay et al. 2002; Spradling et al. 2004), and within this family it appears that the genus *Thomomys* experienced a similar rapid divergence between 3–6 mya (Chapter 4; Belfiore et al. 2008). The objective of this study is to explore ecological niche models (ENMs) in this complex and investigate the roles niche
Fig. 5.1.—Distribution of Thomomys bottae (diagonal lines) and three of the four species in the T. umbrinus species complex in the western United States and Mexico. T. nayarensis is known from only two localities (Chapter 3) and is not included in this analysis. Gray circles (T. bottae) and white circles (T. umbrinus complex) indicate localities used in this study. T. atrovarius and T. sheldoni are recently resurrected species (Hafner et al. 2011; Chapter 2) previously recognized as T. umbrinus. Northern Desert, Central Plateau, and TMVB (Trans-Mexico Volcanic Belt) represent major genetic clades within T. umbrinus. The three boxes indicate the only known sites of contact (or presumed contact) between members of the six mapped groups.
conservatism and competition may have played in shaping and maintaining the current, largely allopatric, species distributions.

5.2 MATERIALS AND METHODS

Data collection.—Capture localities of *T. umbrinus* and *T. bottae* were downloaded from the Mammal Networked Information System (MaNIS; http://manisnet.org). Museum records that did not have GPS coordinates were georeferenced using GEOLocate 3.22 (Rios and Bart 2010). Duplicate records and localities that could not be georeferenced with confidence were omitted from the analysis. Museum records were supplemented with new records of *Thomomys* obtained from recent fieldwork. Because spatially autocorrelated records can lead to inflated measures of accuracy in niche modeling (Veloz 2009), localities included in the analysis were no closer than 10 km apart. When multiple localities occurred within a 10 km radius of each other, those with the lowest quality metadata were removed.

Species distribution modeling.—Nineteen climatic layers, each with a resolution of 1 km², were downloaded from the WorldClim database (Hijmans et al. 2005). These layers represent precipitation and temperature variables compiled from climate stations around the world from 1960–1990 (Hijmans et al. 2005). To avoid over-parameterization of niche models, a correlation analysis of the climatic layers was conducted using ENMTools 1.3 (Warren et al. 2010). Layers that were highly corrected (≥ 0.75) to all other layers were removed from the analysis, leaving 6 uncorrelated climatic layers: annual mean temperature (Bio1); temperature seasonality (Bio4); minimum temperature of coldest month (Bio6); mean temperature of wettest quarter (Bio8); mean temperature of driest quarter (Bio9); and mean temperature of coldest quarter (Bio11). All precipitation layers were highly correlated with one another; annual
precipitation (Bio12) was included in the analysis in order to include at least one uncorrelated precipitation-based layer.

A global digital elevation model (DEM) of North America with a resolution of 1 km$^2$ was clipped to Mexico and the western United States (GTOPO30; available from USGS). The program Spatial Analyst (ArcGIS 10, ESRI, Redwoods, CA) was used to calculate 3 topographic layers from the DEM that may play a role in where pocket gophers are able to establish populations: aspect (compass direction of slope), slope, and water flow accumulation. Because aspect is a circular variable, it was further transformed into 2 linear continuous variables using the sine and cosine of the aspect variable to create easting and northing variables (MacLeod et al. 2008). Pocket gophers are fossorial so 4 soil layers were included in the analysis as possible predictors of distributions. The variables soil type, topsoil texture, reference soil depth, and topsoil reference bulk density were extracted from a 30-second soil raster downloaded from the Harmonized World Soil Database (FAO/IIASA/ISRIC/ISSCAS/JRC 2009). The first 3 variables are categorical layers, and the reference bulk density is continuous.

Species distribution models were created in Maxent 3.3.3e (Phillips et al. 2006), which uses a machine-learning algorithm to generate SDMs from the environmental layers. Pocket gopher locality records for the T. umbrinus species complex were divided into five groups, as follows: T. atrovarius (Hafner et al. 2011); T. sheldoni (Chapter 2); and the three genetic clades comprising T. umbrinus: Central Plateau, Northern Desert, and Trans Mexican Volcanic Belt (TMVB) (Fig. 5.1). The recently described species T. nayarensis was not included in the analysis because it is known from only two localities at present (see Chapter 3).

The ecological models generated in the analysis were used to assess which, if any, environmental factors could be important predictors of the distributions of the five clades. Data
was separated into training and test data: the training data were used to effectively formulate the models and 25% of the training localities (randomly selected) were set aside to test and assess the accuracy of the models. The program was run for 500 iterations for the five *T. umbrinus* groups and for *T. bottae*. Fifty replicates were generated for each of the groups. Model fit was tested using the area under the receiver operating characteristic curve, hereafter referred to as AUC (area under curve). An AUC of 1.0 would indicate the model could perfectly distinguish between presence and absence of the species. Because AUC is correlated with study area and prevalence of occurrence points, it may not necessarily be a good indicator of model fit (Lobo et al. 2008). A second statistic, the true skill statistic (TSS; Allouche et al. 2006), was also calculated. TSS is not affected by prevalence and may be a more unbiased estimate of model fit.

**Niche equivalency and conservatism.**—To assess whether any two groups of *Thomomys* in Mexico had identical or similar niche tolerances, the ENMTools program was used to generate niche overlap statistics and test hypotheses of niche equivalency and conservatism among the Mexican *Thomomys*. Niche overlap statistics measure the degree of habitat similarity between groups by comparing their SDMs projected onto the environmental layers found in the region of interest (Warren et al. 2008). Two empirical metrics of overlap were calculated: Schoener’s D (Schoener 1968) and the *I* statistic developed by Warren et al. (2008). These statistics range from 0 (completely distinct SDMs) to 1 (identical SDMs). To test for niche equivalency, identity tests were run for all pair-wise comparisons among the 5 groups. These tests pool occurrence points between any 2 groups, remove and randomize their identities, and then produce new measures of overlap using the same number of observations as the empirical data. This is done for 100 pseudoreplicates, forming a null distribution to compare with the empirically calculated overlap
statistics. A one-tailed test evaluates the null hypothesis that the habitat suitability models for each pair-wise comparison are not significantly different (Warren et al. 2008).

The niche identity test can be a very strict assessment of overlap, requiring sets of species or groups to be exposed to and tolerate the same bioclimatic variables; not necessarily true for allopatric species. Niche similarity may be a more realistic assessment of whether any 2 species’ bioclimatic niche spaces are more conserved than one would expect by chance. Background tests use a randomization procedure to evaluate whether any two species or groups had habitat suitability scores that were more or less similar based on the availability of various geographic areas (or “backgrounds”) where they could potentially occur (Warren et al. 2008). This is done by comparing a set of empirically known occurrence points from one group (“Species A”) to the same number of points randomly drawn from a specified background representing another group (“Species B”). The two sets of habitat suitability scores are then measured for overlap much in the same way as the identity test. Each background test is run for 100 pseudoreplicates, generating a null distribution.

Whereas the identity test is one-tailed, the background test is two-tailed and tests the null hypothesis that Species A’s habitat suitability scores are not significantly more or less similar than expected by chance. If the empirical overlap statistic falls outside to the right of the null distribution, then Species A’s SDM is considered significantly more similar to the chosen background; an overlap that is to the left of the distribution is significantly less similar. The niche conservatism test is then reversed so that Species B’s known localities are compared to random points from Species A’s background.

Because these similarity tests are a function of the background used, I chose four different types of backgrounds to test for evidence of conservatism (Fig. 5.2). These backgrounds
Fig. 5.2.—Schematic showing the 4 types of background tests performed on species of the *Thomomys umbrinus* complex to test niche similarity. This example shows *T. sheldoni* being compared to various backgrounds for the Central Plateau (CP) clade of *T. umbrinus*. A) *T. sheldoni* localities compared to the known minimum range of CP; B) *T. sheldoni* localities compared to a MaxEnt prediction for CP, constrained to only 50% or greater probability of occurrence; C) *T. sheldoni* localities compared to a 5 km buffer zone around known CP localities; and D) *T. sheldoni* localities compared to a background of all possible habitats except that occupied by CP.
were chosen to represent various landscape-level scales, in an effort to judge at what level and to what degree bioclimatic envelopes are similar between any two groups. The backgrounds chosen were as follows: the known minimum distribution of a species or clade, visualized as a minimum convex polygon (Fig 5.2a); a SDM generated in MaxEnt, clipped to predictions 50% or greater, representing the potential “core range” of a species (Fig. 5.2b); and a five km buffer zone around known occurrence points, representing a population-level analysis (Fig. 5.2c). A fourth background was also created to represent possible competitive exclusion. In this background, the only area that is not available to a species is the known minimum distribution of a possible competitor (Fig. 5.2d); if Species A has a significantly similar bioclimatic envelope to all potential surrounding areas, than I could presume that possibly competitive exclusion can explain the current distribution. Niche similarity tests were only performed between groups that had neighboring distributions, excluding groups that had no potential to come into contact, such as T. atrovarius and the Central Plateau T. umbrinus.

5.3 RESULTS

Species distribution models.—To generate the SDMs, 215 records were used for the T. umbrinus group (45 T. atrovarius, 40 T. shelgoni, 38 T. umbrinus [Central Plateau], 24 T. umbrinus [Northern Desert], and 68 T. umbrinus [TMVB]) and 768 records were used for T. bottae (Figs. 5.1 and 5.3). All six models displayed relatively high fit, with average AUC values ≥ 0.96 on the test data for the five groups in the T. umbrinus species complex and AUC = 0.86 for T. bottae (Table 5.1). TSS values were lower than the AUC values, ranging from 0.32 to 0.66. For 3 models (T. atrovarius, T. shelgoni, and TMVB T. umbrinus), TSS was ≥ 0.6, indicating good model fit. The remaining 3 models had poor fit, with TSS ≤ 0.4. Jackknife tests were run to assess the importance of individual variables in each of the SDMs. Temperature
Fig. 5.3.—Predicted species distributions generated in MaxEnt for the *Thomomys umbrinus* species complex. White circles indicate museum records used to generate distributions. Scale bars within each graphic indicate the probability of predicted occurrence.
Table 5.1.—Area under the receiver operating characteristic curves (area under curve, AUC) and the true skill statistic (TSS) are presented as indicators of model fit. AUC is given for training data (used to formulate the model parameters) and test data (used to assess accuracy of the model) averaged over 50 replicates with standard deviation, generated from MaxEnt analyses. Bioclimatic variables that had the greatest overall percent contribution to the model and greatest permutation importance are listed with their percentages for each model.

<table>
<thead>
<tr>
<th>Species</th>
<th>Training AUC</th>
<th>Test AUC</th>
<th>TSS</th>
<th>Percent contribution</th>
<th>Permutation importance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. atrovarius</em></td>
<td>0.994</td>
<td>0.978 ± 0.013</td>
<td>0.63</td>
<td>Mean temp coldest quarter</td>
<td>27.89</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td>0.994</td>
<td>0.988 ± 0.005</td>
<td>0.66</td>
<td>Soil depth</td>
<td>35.85</td>
</tr>
<tr>
<td><em>T. umbrinus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Desert</td>
<td>0.997</td>
<td>0.985 ± 0.006</td>
<td>0.39</td>
<td>Temperature seasonality</td>
<td>31.54</td>
</tr>
<tr>
<td>Central Plateau</td>
<td>0.992</td>
<td>0.960 ± 0.024</td>
<td>0.32</td>
<td>Temperature seasonality</td>
<td>43.75</td>
</tr>
<tr>
<td>TMVB</td>
<td>0.988</td>
<td>0.978 ± 0.006</td>
<td>0.60</td>
<td>Temperature seasonality</td>
<td>64.82</td>
</tr>
<tr>
<td><em>T. bottae</em></td>
<td>0.88</td>
<td>0.852 ± 0.01</td>
<td>0.40</td>
<td>Temperature seasonality</td>
<td>22.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean temp coldest quarter</td>
<td>25.71</td>
</tr>
</tbody>
</table>
seasonality was the largest contributor to the models for all but *T. atrovarius* and *T. sheldoni*. Temperature seasonality also had the greatest permutation importance (the importance in the final model) for all models except that of *T. bottae* (Table 5.1).

*Niche identity tests.*—Niche overlap as measured by Schoener’s D ranged from a low of 0.04 between *T. umbrinus* Northern Desert and *T. atrovarius* to a high of 0.53 between *T. umbrinus* Central Plateau and *T. sheldoni* (Table 5.2). Niche overlap measured using the I statistic ranged from 0.11 between *T. umbrinus* Northern Desert and *T. atrovarius* to 0.60 between *T. umbrinus* Central Plateau and *T. umbrinus* Northern Desert (Table 5.2). All empirical overlap values fell outside the null distributions created from 100 pseudoreplicates for each pairwise comparison in the identity tests, indicating that all models were significantly different from each other and rejecting the null hypothesis of niche equivalency.

*Niche conservatism tests.*—When one species’ known occurrences were compared to the minimum known range of another species, *T. sheldoni* and Northern Desert *T. umbrinus* both had habitat suitability scores more similar to each other than would be expected by chance (Table 5.3). *T. atrovarius* was more similar when projected onto the background of the *T. bottae* known range but less similar when compared to the known range of *T. sheldoni* for Schoener’s D.

When a species was compared to what may represent the possible “core” range of another species, predicted from the SDMs constrained to only 50% or greater prediction of occurrence, the empirical SDMs of *T. sheldoni* and *T. atrovarius* individuals were both more similar to the other’s core range (Table 5.3). *T. atrovarius* and TMVB *T. umbrinus* were also more similar for this comparison. Central Plateau *T. umbrinus* and TMVB *T. umbrinus* did have a more similar bioclimatic niche compared to the core range of *T. sheldoni*, as did Northern Desert *T. umbrinus*.
Table 5.2.—Niche overlap results for Mexican *Thomomys*. Schoener’s D is shown above the diagonal, the *I* statistic below. Values of both statistics range from 0 (completely distinct niche models) to 1.0 (identical niche models).

<table>
<thead>
<tr>
<th></th>
<th><em>T. atrovarius</em></th>
<th><em>T. sheldoni</em></th>
<th><em>T. umbrinus</em></th>
<th><em>T. bottae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. atrovarius</em></td>
<td>1</td>
<td>0.34</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td>0.14</td>
<td>1</td>
<td>0.53</td>
<td>0.48</td>
</tr>
<tr>
<td><em>T. umbrinus</em></td>
<td></td>
<td></td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Central Plateau</td>
<td>0.15</td>
<td>0.53</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Northern Desert</td>
<td>0.11</td>
<td>0.48</td>
<td>0.60</td>
<td>0.30</td>
</tr>
<tr>
<td>TMVB</td>
<td>0.44</td>
<td>0.57</td>
<td>0.39</td>
<td>0.19</td>
</tr>
<tr>
<td><em>T. bottae</em></td>
<td>0.28</td>
<td>0.41</td>
<td>0.53</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 5.3.—Background test results of niche conservation for each comparison of genetic clades within the *T. umbrinus* species complex between adjoining allopatric or parapatric distributions. Tests are species A is being projected onto the background of species B and then reversed. The 3 background tests presented are the known minimum range of species B ("Opposite Ranges"), the possible core range of species B as predicted by MaxEnt where species probability of occurrence was ≥ 50% ("MaxEnt 50"), and all available habitat with the exception of the known range of species B ("Clipped Out Range"). When single result is presented, both the *I* statistic and Schoener’s D were the same. When they differed, results are presented as *I* statistic/Schoener’s D. NS = non-significant; More = more similar; Less = less similar. ND = Northern Desert, CP = Central Plateau, TMVB = Trans-Mexico Volcanic Belt.

<table>
<thead>
<tr>
<th>Species A</th>
<th>Species B</th>
<th>Opposite ranges</th>
<th>MaxEnt 50</th>
<th>Clipped out range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. sheldoni</em></td>
<td><em>T. atrovarius</em></td>
<td>NS</td>
<td>More</td>
<td>Less/NS</td>
</tr>
<tr>
<td><em>T. atrovarius</em></td>
<td><em>T. sheldoni</em></td>
<td>NS/Less</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td><em>T. umbrinus</em> ND</td>
<td>More</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>T. umbrinus</em> ND</td>
<td><em>T. sheldoni</em></td>
<td>More</td>
<td>NS</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td><em>T. umbrinus</em> CP</td>
<td>NS</td>
<td>NS</td>
<td>More</td>
</tr>
<tr>
<td><em>T. umbrinus</em> CP</td>
<td><em>T. sheldoni</em></td>
<td>NS</td>
<td>More</td>
<td>Less/NS</td>
</tr>
<tr>
<td><em>T. sheldoni</em></td>
<td><em>T. umbrinus</em> TMVB</td>
<td>NS</td>
<td>NS</td>
<td>More</td>
</tr>
<tr>
<td><em>T. umbrinus</em> TMVB</td>
<td><em>T. sheldoni</em></td>
<td>NS/Less</td>
<td>NS/More</td>
<td>More</td>
</tr>
<tr>
<td><em>T. atrovarius</em></td>
<td><em>T. umbrinus</em> ND</td>
<td>NS</td>
<td>NS</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. umbrinus</em> ND</td>
<td><em>T. atrovarius</em></td>
<td>NS</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. atrovarius</em></td>
<td><em>T. umbrinus</em> TMVB</td>
<td>NS</td>
<td>More</td>
<td>NS</td>
</tr>
<tr>
<td><em>T. umbrinus</em> TMVB</td>
<td><em>T. atrovarius</em></td>
<td>NS</td>
<td>More</td>
<td>NS</td>
</tr>
<tr>
<td><em>T. atrovarius</em></td>
<td><em>T. bottae</em></td>
<td>More</td>
<td>NS</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. bottae</em></td>
<td><em>T. atrovarius</em></td>
<td>NS</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. umbrinus</em> ND</td>
<td><em>T. bottae</em></td>
<td>NS</td>
<td>NS</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. bottae</em></td>
<td><em>T. umbrinus</em> ND</td>
<td>NS</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. umbrinus</em> CP</td>
<td><em>T. umbrinus</em> ND</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>T. umbrinus</em> ND</td>
<td><em>T. umbrinus</em> CP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>T. umbrinus</em> CP</td>
<td><em>T. umbrinus</em> TMVB</td>
<td>NS</td>
<td>NS</td>
<td>Less</td>
</tr>
<tr>
<td><em>T. umbrinus</em> TMVB</td>
<td><em>T. umbrinus</em> CP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

when compared to the *T. atrovarius* core range; however these three comparisons did not hold up when the test was reversed. *T. bottae* individuals were also more similar to random points generated in the *T. atrovarius* and Northern Desert *T. umbrinus* core ranges. Using a five km buffer around known localities resulted in non-significant tests for all comparisons (not shown).
When a species was compared to all available niche space except that potentially occupied by “Species B”, *T. atrovarius* tended to be less similar to all other available niche space when compared to the three of the four species with which it approaches parapatry or sympatry. No significant difference was found when compared to TVMB *T. umbrinus* (Table 5.3). *T. sheldoni* tended to be less similar to this available niche space for at least one of the statistics when compared against Northern Desert and Central Plateau *T. umbrinus*, but was more similar when compared against TMVB *T. umbrinus*. *T. bottae* was also less similar to the available niche space other than that occupied by *T. atrovarius* and Northern Desert *T. umbrinus*.

5.4 DISCUSSION

Fossorial animals present unusual challenges to species distribution modeling because they are buffered to some extent from the external environment and live in a relatively stable space (Nevo 1979). Fossorial niches also tend to favor species with low vagility, possibly decreasing their potential to fill available niche space. While it would appear that soil depth and texture would be important limiting factors for fossorial animals, there are likely many interacting biotic and abiotic factors that interact in shaping how fossorial species arrange themselves on the landscape (Munguía et al. 2008).

Modeling known localities using environmental variables resulted in fairly accurate predictions for *Thomomys*, despite their fossorial lifestyle. All the models had AUC values above 0.98, except *T. bottae*. Species with narrow ranges, such as most representatives within the *T. umbrinus* complex, may have higher, artificially inflated AUC values (Lobo et al. 2007). The TSS, a more unbiased measure, had moderately high values for *T. atrovarius* and *T. sheldoni*, an indication of good model fit. TMVB *T. umbrinus* had between fair and good fit and the remaining groups had relatively poor predictions and poor model fit, according to the TSS.
The climatic envelope for *T. atrovarius* had been previously modeled and these gophers had significant habitat differences when compared to the some other members of the complex (Hafner et al. 2011). *T. atrovarius* is found primarily at low elevations in dry, thornscrub vegetation along the Pacific coast of Sinaloa and Nayarit. In contrast, *T. sheldoni* is restricted to high elevation (> 2,000 m) pine-oak woodlands in the Sierra Madre Occidental (Chapter 2). The relatively specialized niche of *T. sheldoni* likely results from an upward shift in distribution in response to periodic climate warming that led to genetic isolation from ancestral *T. atrovarius* stock and subsequent niche specialization (Escalante et al. 2004; 2007).

Although temperature seasonality (SD of mean monthly temperatures x 100) contributed most heavily to the SDMs for the 3 genetic clades of *T. umbrinus*, the SDM of *T. atrovarius* was influenced most by mean temperature at the coldest quarter and that of *T. sheldoni* by soil depth. Populations of *T. atrovarius* occur almost exclusively at low elevations, and they may be unable to tolerate the seasonally cold temperatures at higher elevations. In contrast, populations of *T. sheldoni* are restricted to higher elevation habitats in the Sierra Madre Occidental, where soil erosion can be high (Descroix et al. 2001; 2008), putting soil depth sufficient for burrowing at a premium.

The hypothesis of niche equivalency was rejected for all pair-wise tests in this study. However, in view of the allopatric distributions of the groups in this species complex, this was not surprising, as they would have to be exposed to the same environmental conditions in order to be equivalent for this test (Warren et al. 2008). Although we have no information on potential competitive interactions among *Thomomys* populations in Mexico, the relatively low level of niche overlap measured in this study are consistent with Pianka’s (1974) niche overlap hypothesis, which states that niche overlap should vary inversely with increasing competition.
Yet the strong allopatry seen in this complex implies competition may not currently be important, except in areas of sympatry or between more distantly related geomyids.

*Thomomys umbrinus*, which has a broadest distribution of all Mexican *Thomomys*, appears to tolerate a much wider range of elevations and habitat types. This species is found up to 4,000 m, and is frequently found in agricultural areas and open or disturbed habitats. MaxEnt predictions for all 3 genetic clades of *T. umbrinus* were much broader than predictions for *T. atrovarius* and *T. sheldoni* (Fig. 5.3), and fairly high predictions existed in areas where no *Thomomys* populations occur. Most of these areas are currently inhabited by pocket gophers of other genera (*Cratogeomys*, *Orthogeomys*, or *Pappogeomys*) who, by virtue of their larger body sizes, may be competitively dominant to *T. umbrinus*. *T. umbrinus* is also predicted to be in areas presently occupied by *T. bottae*, an aggressive competitor that tends to displace *T. umbrinus* whenever the 2 species come into contact (Best 1973; Miller 1964; Patton and Dingman 1968). Thus, competition may be excluding *T. umbrinus* from many areas of suitable habitat in Mexico and the southwestern United States (Fig. 5.3).

Using the “clipped out range” (Fig. 5.2d) was meant to simulate an environment where a species could theoretically inhabit any area except that occupied by a potential competitor, thus possibly implying that the presence of competitors are shaping current distributions more so than climatic factors. When *T. sheldoni* and TMVB *T. umbrinus* were used as each other’s competitor, there was a higher degree of similarity detected to all other available habitat. This group of *T. umbrinus* tends to inhabit higher elevations, specifically within the TMVB region, which is similar to the *T. sheldoni* elevation requirement, so it is possible that *T. sheldoni* could expand into the TMVB region if given the opportunity. Other than this comparison, the lack of similarity among niche space seems to imply that competition is not an important factor within the *T.*
The *umbrinus* complex, or at least was not detectable at the scale studied. It appears to be more important between more distantly related species such as *T. bottae* or *Cratogeomys* species where they do experience higher degrees of parapatry and sympathy.

Interspecific competition can only play a role where members of the *T. umbrinus* complex approach sympatry, such as between *T. sheldoni* and *T. umbrinus* in the Northern Desert clade. These two species showed evidence of niche conservatism when their overall ranges were compared. This may be explained by the fact that they have the closest distributions within the *T. umbrinus* complex, known from within two km of one another in northwestern Chihuahua. They likely do come into contact, although it has not yet been documented. MaxEnt over-predicts Northern Desert *T. umbrinus* to occur farther south into the territory of *T. sheldoni*. It is unknown whether *T. sheldoni* already inhabited the pine-oak woodlands in northwestern Chihuahua when the two species came into secondary contact, but *T. sheldoni* may be a superior competitor that has prevented *T. umbrinus* from expanding into its range.

Other examples of niche conservatism were found when only the highest predicted distributions were used as a background (Fig. 5.2b), at the possible core of their ranges. *Thomomys atrovarius* is considered basal to the complex, diverging from *T. bottae* sometime between 2 and 4 mya (Chapter 4; Belfiore et al. 2008). The southern Sierra Madre Occidental is currently the area of highest species diversity in the *T. umbrinus* species complex, with all 4 species found within 60 km of one another. If we assume that this was the site of initial diversification of the group, this may account for the niche conservatism measured at what would be the “core” ranges of the species. Despite this possible relictual conservatism, it is apparent that the niches of these species are not similar, indicating that despite their close phylogenetic relatedness, ecologically they are more distinct than expected (Losos 2008).
Because the predicted climatic envelopes of *T. atrovarius* and *T. sheldoni* generally fall outside those of the 3 *T. umbrinus* clades (Fig 5.3), we can infer that past niche evolution and current niche conservatism likely plays an important role in maintaining the distributions of these two species (Wiens and Graham 2005). The absence of strong niche differentiation among the 3 *T. umbrinus* clades suggests that niche evolution did not play an important role in the divergence of these clades (Nakazato et al. 2010), a fact that further strengthens the argument that these genetic groups should be considered conspecific (see Chapter 4). Despite the seemingly homogeneous nature of their fossorial lifestyle, it is obvious that ecological partitioning does play a stronger role among these closely related rodents in maintaining the allopatric distributions we see today rather than competition.
Adaptive radiations are inherently fascinating due to the ability of new species to evolve and fill a previously unutilized niche space. Yet non-adaptive radiations, such as investigated in salamanders (Kozak et al. 2006; Wake 2006) and snails (Holland and Hadfield 2004), can be equally interesting when one considers that these radiations may involve multiple new species evolving, often in a relatively brief period of time, to theoretically compete amongst themselves. I began this dissertation with the primary goal of exploring presumed speciation processes in a poorly studied species of pocket gopher, *Thomomys umbrinus*, known to have 2 different diploid numbers and 5 genetic clades. Patton and Feder (1978) and Hafner et al. (1987) both suggested multiple species might be present within *T. umbrinus*. While some recent collections of *T. umbrinus* were made in the 1990’s and a few in 2005 in Mexico, this species had not been the focus of any research in almost 20 years and no multi-locus phylogenetic analyses had been published.

Using molecular analyses and cranial morphology, I have demonstrated that the Northern and Southern Sierra Madre clades, thought to possibly be genetically distinct, are not distinct at the species level, although low levels of genetic differentiation and differences in cranial morphology did warrant subspecies-level separation. However, the combined Sierra Madre clades are genetically isolated from *T. umbrinus*. No evidence of gene flow was detected between *T. sheldoni* and *T. umbrinus*, despite finding populations within 2 km of one another. Accordingly, I formally described this taxon as *T. sheldoni*.

Through the sampling of newly collected individuals of *T. umbrinus*, I discovered a new member of the *T. umbrinus* complex, formally named and described herein as *T. nayarensis*. This new species is only known from only 2 localities in the Sierra del Nayar of northeastern Nayarit.
and appears to have a highly restricted distribution. *T. nayarensis* was a surprising find, nestled between populations of *T. sheldoni* and *T. atrovarius*, within 12 km of both with no evidence of gene flow. Although cranial morphology is largely conserved among the 3 species in this region, 2 measurements (width and length of the auditory meatus) proved to be useful in distinguishing between specimens of *T. sheldoni* and *T. nayarensis*.

I demonstrated that although 3 genetic clades within *T. umbrinus* (2n = 78) do have high levels of genetic differentiation between them (approximately 13%–16% at cytochrome b [Cytb]), they should be considered the same species due to shared nuclear haplotypes, shared diploid number, no discordance in allozyme alleles, and no real evidence that they are geographically isolated. Species tree analyses coupled with traditional concatenated analyses aided in resolving the many subspecies found within *T. umbrinus*, reducing them from 15 to 4. A clear justification for subspecies level recognition was provided; one that is much more reliable than body size or pelage characters, which are notoriously variable in pocket gophers.

Finally, I explored the species distributions within this group of rodents, using modeling techniques to assess what, if any, biotic factors were important in shaping or maintaining the distributions of each of these taxa. I showed that temperature seasonality was important for predicting the distribution of the 3 genetic clades of *T. umbrinus*, *T. atrovarius* and *T. sheldoni* each had a different variable that proved important in shaping their distributions: mean temperature of coldest quarter and soil depth, respectively. I also demonstrated that past niche evolution in *T. atrovarius* and *T. sheldoni* and current niche conservatism has likely shaped their current distributions and competition between *T. sheldoni* and *T. umbrinus* in northwestern Chihuahua is likely preventing any sympatry between these species.
In sum, 124 individuals representing the 4 species of the *T. umbrinus* complex and *T. bottae* were collected between 2006 and 2012; many from new localities and almost all representing never-before collected genetic material (Fig. 6.1). Of these individuals, 17 were sequenced for only *Cytb* and the remaining 54 were sequenced for up to 3 mitochondrial and 5 nuclear genes. Representatives of 2 other species used as outgroups in the phylogenetic analyses, *Orthogeomys hispidus* and *T. talpoides*, were also sequenced for these 8 genes. For the morphological studies, 344 individuals were measured for 12 cranial variables. What once was considered a single species, *T. umbrinus*, with 25 subspecies originally described based on morphology, is now recognized as 4 species representing 8 subspecies described based on genetic evidence supplemented by cranial morphometric data.

Throughout the course of this study, I have clarified and delimited the distribution of each species in this complex to the best of my current knowledge. More intensive sampling, especially in the regions where sampling has been poor or no efforts made, will aid us in understanding the true ranges of these species. Population-level studies are also needed as they are largely absent in this species complex. This is especially true for *T. nayarensis*, whose extremely restricted distribution and close proximity to other *Thomomys* species speaks to the need for more research about their possible interactions. Ecological studies at the population level would also shed light on the habitat requirements of these 4 species. With the continued advances in molecular methods, highly divergent groups that have poor phylogenetic resolution at the basal nodes, such as this one, may have an opportunity to be clarified in the future. Such findings will only enhance our knowledge of how species evolve and persist.
Fig. 6.1. Generalized distributions of the 4 species in the *Thomomys umbrinus* complex in Mexico and the southwestern United States, with the newly recognized subspecies shown in gray. Black circles represent localities of genetic samples collected prior to 2006 and white circles are those collected in the course of this study (2006–2012). Gray circles are ancient DNA samples used in Chapters 2 and 3 of this dissertation.
LITERATURE CITED


RIOS, N. E., AND H. L. BART. 2010. GEOLocate (Version 3.22) Tulane University Museum of Natural History, Belle Chasse, LA.


APPENDIX 2.1
LIST OF SPECIMENS EXAMINED IN CHAPTER 2

Specimens new to Chapter 2 are deposited in the Collection of Mammals, Louisiana State University Museum of Natural Science (LSUMZ). Other specimens used in this study are housed in the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), the United States National Museum of Natural History (USNM), the University of Kansas Natural History Museum (KU), Colección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México (CNMA), the Museum of Southwestern Biology, University of New Mexico (MSB), the California Academy of Sciences (CAS), and the New Mexico Museum of Natural History (NMMNH). Specimens used in the molecular analyses are designated “M” (“aM” for ancient DNA), those used in the chromosomal analysis are designated “k”, those used in the morphometric analyses are designated “m”, and those in the allozyme analyses are designated “a.” GenBank numbers for DNA sequences are given in Appendix 2.3. Sample sizes for each kind of analysis are indicated following the taxon names. Boldface numbers in parentheses before locality names refer to mapped localities in Fig. 2.1. Localities are listed north to south within states.

*T. sheldoni chihuahuae* (M = 13, m = 24, k = 22, a = 91)

**MEXICO: Chihuahua; (7)** 3.5 km S, 4.5 km E Colonia Garcia, 2,300 m (29.945, -108.289), LSUMZ 36731 (M, m, k), LSUMZ 36732 (m, k); (6) 4 km S, 1 km E Colonia Garcia, 2,200 m (29.937, -108.327), LSUMZ 36723 (M, m, k); 5.5 km S, 5.5 km E Colonia Garcia, 2,320 m (29.927, -108.283), LSUMZ 36739 (M, m, k), LSUMZ 36733–38 (m, k); 8 km S, 4 km E Colonia Garcia, 2,323 m (29.903, -108.3), LSUMZ 36740 (M, k); 11 km S, 3 km E Colonia Garcia, 2,200 m (29.879, -108.309), LSUMZ 36724 (M, k), LSUMZ 36725–26 (m, k); Valle Moctezuma, 11.6 mi. SE (by road) Colonia Garcia (29.833, -108.274), MVZ 150582 (M, k),
MVZ 150571–150584 (a); 1.3 mi. E (by road) Chuhuichupa (29.624, -108.362), MVZ 150565 (M, k), MVZ 150544–70 (a); 1.5 mi. NE (by road) Madera (29.22, -108.10), MVZ 150538–43 (a); 9.6 mi. W (by road) Tomochic (28.360, -107.94), MVZ 150512 (M, k), MVZ 150510–20 (a); Rancho El Pajarito, 25.0 mi. W (by road) Tomochic (28.234, -108.081), MVZ 150526 (M, k), MVZ 150521–37 (a); 9.6 mi. W (by road) Tomochic (28.360, -107.94), MVZ 150512 (M, k), MVZ 150510–20 (a); Rancho El Pajarito, 25.0 mi. W (by road) Tomochic (28.234, -108.081), MVZ 150526 (M, k), MVZ 150521–37 (a); (12) 5 km SE Creel, 2,033 m (27.714, -107.608), LSUMZ 36696 (M, m, k); (13) La Laja, 10 km SE Samachique, 2,500 m (27.269, -107.446), LSUMZ 36700 (M, m, k), LSUMZ 36698–99 (m, k); Sierra Madre, 65 mi. E of Batopilas (27.023, -106.691), USNM 96455–56 (m); (15) 6.5 km N, 7 km E El Vergel, 2,712 m (26.538, -106.315), LSUMZ 36742 (M, k), LSUMZ 36743 (m, k); 1.8 mi. E (by road) El Vergel (26.476, -106.357), MVZ 150481 (M, k), MVZ 150475–90 (a); Sierra Madre, near Guadalupe Y Calvo (26.089, -106.965), USNM 95247–48, 95251–52 (m)

T. s. sheldoni (M = 8, aM = 4, m = 30, k = 7, a = 45)

MEXICO: Durango; 22 mi. WSW Durango, 7,900 ft (23.92, -104.98), CAS 12277 (m), 12278 (aM, m); 1.3 mi. NE Mil Diez [= Mil Dias] (23.812, -105.373), MVZ 147068 (M, k), MVZ 147061–82 (a); 12 km E El Salto, 2,490 m (23.783, -105.239), LSUMZ 34354 (M); El Salto (23.779, -105.361), USNM 946111, 94647–94 (m); (21) 1 mi. E La Ciudad, 2,590 m (23.732, -105.676), MVZ 150444 (M, k), MVZ 150425–47 (a); (22) 33 km S, 7 km W Durango, 2,420 m (23.707, -104.732), LSUMZ 36811 (M, m, k); 43 km S, 32 km W Vincente Guerrero, 2,600 m (23.339, -104.291), LSUMZ 36810 (M, m, k); Nayarit; Santa Teresa, 6,800 ft. (22.488, -104.753), USNM 523456 (aM, m), USNM 523468 (aM), USNM 90823, 90826 (m); Santa Teresa, 13 km SW; Rancho Viejo (22.409, -104.835), USNM 523458–59, 523463 (m), USNM 523465 (aM, m); 3 km (by road) SE Santa Gertrudis, 2,360 m (22.376, -104.811), LSUMZ 36831 (M, m), LSUMZ 36834 (m); Zacatecas; 8 mi. W Milpillas, 60 mi. W Fresnillo, 8,300 ft.
(23.07, -103.8), CAS 11083 (m); 8 mi. S Chalchuites (= Chalchihuites), 8,600 ft. (23.36, -103.88), CAS 13000, 13162–63 (m); (24) 6 km N, 15 km W Valparaiso, 2,730 m (22.827, -103.72), LSUMZ 36804 (M, m, k), LSUMZ 36805 (m, k), LSUMZ 36806 (m); Valparaiso Mountains (22.827, -103.72), USNM 91985, 91987, 91994 (m); Sierra Madre (22.601, -104.333), USNM 90830–32 (m); (27) Santa Cruz, 2,480 m (22.41, -104.346), LSUMZ 36800 (M, k)

*T. umbrinus* (M = 39, aM = 1, m = 48, k = 40, a = 102)

**ARIZONA: Santa Cruz Co.**; (1) Sycamore Canyon, Patagonia Mountains, 1,341 m (31.386, -110.743), MVZ 148307 (M, k), MVZ 148306–318 (a); **NEW MEXICO: Hidalgo Co.**; (2) Animas Mountains, 5.2 mi. N, 8.7 mi. W Hilo Peak (31.472, -108.747), NMMNH 1920 (M); **MEXICO: Chihuahua;** Río El Gavilán, 7 mi. SW Pacheco (30.015, -108.417), MVZ 109657–58, 109661–62, 109664, 109668, 109670 (m); near Colonia Garcia (= 10 mi. NE Colonia Garcia, Pilares Canyon; Anderson 1972; 30.08, -108.21), USNM 98204–05, 98208 (m); 2.4 mi. NE (by road) Colonia Garcia (30.002, -108.32), MVZ 150606 (M, k), MVZ 150585–610 (a); (5) 2 km S, 0.5 km E Colonia Garcia, 2,200 m (29.958, -108.333), LSUMZ 36721 (M, k); (4) 6 km E Colonia Garcia, 2,200 m (29.974, -108.275), LSUMZ 36728 (M, k), LSUMZ 36727, 26729–30 (m, k); (8) Cañón del Arroyo Santa Clara, Sierra del Nido (29.366, -106.572), MVZ 147083 (M, k); (9) 9 km N Santo Tomas, 2,100 m (28.731, -107.648), LSUMZ 36694 (M, m); (10) 8.4 mi. W (by road) Cuauhtémoc (28.387, -107.006), MVZ 150508 (M, k); (11) 10 km N, 5 km E MEOQUI, 1,160 m (28.316, -105.431), LSUMZ 36719 (M, m, k); 5 km S Ciudad Camargo, 1,280 m (27.628, -105.121), LSUMZ 36718 (M, k); (14) Río Belleza, 20 km N, 17 km E El Vergel, 1,730 m (26.655, -106.22), LSUMZ 36745 (m, k), LSUMZ 36747 (M, m, k); **Coahuila;** (19) 15 km (by road) NW La Flor de Jimulco (at km 11 road marker), 1,230 m (25.225, -103.448), LSUMZ
36602 (M); **Durango;** 1 km SE El Ojito, 2,250 m (26.731, -106.043), LSUMZ 36701–02 (m, k); 6 km S, 13 km W El Ojito, 1,770 m (26.683, -106.186), LSUMZ 36741 (M, k); 13 km S, 15 km E El Ojito (26.615, -105.864), LSUMZ 36703 (m, k); 14.7 mi N (by road) Las Nieves (26.537, -105.492), MVZ 150470 (M, k); 10 km N, 20 km W Ocampo, 1,800 m (26.535, -105.711), LSUMZ 36705 (M, k); LSUMZ 36706–08 (m, k); 3 km W Ocampo, 1,750 m (26.459, -105.543), LSUMZ 36709 (M, k), LSUMZ 36710 (m, k); (17) 50 km N, 20 km W Bermejillo, 1,140 m (26.34, -103.803), LSUMZ 34351 (M); 2 km S, 8 km E El Palmito, 1,500 m (25.597, -104.925), LSUMZ 36812 (M, k); (18) Rio Nazas, 2 km S, 29 km E Rodeo, 1,277 m (25.157, -104.266), LSUMZ 36813 (M, k); Durango (24.03, -104.67), USNM 94605, 94607 (m); La Boca del Mezquital, 1,900 m (23.774, -104.445), LSUMZ 36807 (M, m, k), 36808–09 (m, k); (20) 1.5 mi. S (by road) Morcillo (23.732, -105.676), MVZ 150455 (M); **Mexico;** 34 road km E Zitácuaro, (Bosencheve; 19.416, -100.124), LSUMZ 25101 (M); 25 km N Valle de Bravo, 2438 m (19.422, -100.129), LSUMZ 36074; (M); Volcan Iztaccíhuatl, 4 km N Paso de Cortez, 3,842 m (19.064, -98.383), CNMA 42505 (M); **Michoacán;** (33) 6.5 km S Pátzcuaro, 2,200 m (19.421, -101.609), LSUMZ 34359 (M); **Nayarit;** (25) 8.5 km N, 7 km W Mesa del Nayar (formerly listed by Hafner et al. [2011] as “22 km S, 3 km E Santa Teresa”), 2,200 m (22.29, -104.721), LSUMZ 36750 (M), LSUMZ 36796 (m, k), LSUMZ 36751–52 (m); Mesa del Nayar, 4,500 ft. (22.197, -104.65), USNM 51160–64 (m); (26) 1 km S Mesa del Nayar, 1,290 m (22.197, -104.65), LSUMZ 36830 (M, m, k); **Puebla;** (34) Boca del Monte, 3.5 km S, 3 km E Esperanza, 2,450 m (18.83, -97.328), MVZ 153877 (M, k); **San Luis Potosí;** (32) Ventura (22.26, -100.88), MVZ 153799 (M, k); 11 km N, 12 km E Arriaga (21.891, -101.383), MVZ 153810 (M); **Sonora;** E bank of Rio Yaqui at El Novillo (28.98, -109.63), MVZ 148888–99 (a); W bank of Rio Yaqui at El Novillo (28.98, -109.63), MVZ 148900–08 (a); (3) 1 mi. S Moctezuma (29.802, -109.667), MVZ 147097 (M, k),
MVZ 147085–101, 148869–75 (a); 30 km SW Moctezuma, 1,000 m (29.4, -109.5), MSB 61113 (M); Bacanora (28.98, -109.4), MVZ 148876–87 (a); ca. 1 mi. N (by road) Sahuaripa (29.07, -109.24), MVZ 148909–14 (a); Zacatecas; 7 km S, 8 km E Jiménez de Teul, 2,450 m (23.214, -103.737), LSUMZ 36713 (M, k), LSUMZ 36714 (m, k); 3 km N Ojocaliente, 2,030 m (22.597, -102.251), MVZ 153778 (M, k); (28) 4 km N, 3.5 km W Monte Escobedo, 2,430 m (22.342, -103.599), LSUMZ 36801 (M, k), LSUMZ 36802 (m, k), LSUMZ 36803 (m); Plateado (21.95, -103.1), USNM 90837 (m); (31) 5 km S, 18 km E Jalpa, 2,550 m (21.605, -102.836), LSUMZ 36712 (M, m, k); 2.5 mi. N Moyahua, 4,400 ft. (21.3, -103.16), CAS 11082 (aM, m) T. atrovarius (M = 3, aM = 2, m = 22, k = 2)

MEXICO: Durango; 1 mi. SW Revolcaderos (23.6, -105.85), USNM 375708 (m); Jalisco; (29) 6 km N, 12 km W Bolaños, 2,400 m (21.92, -103.893), LSUMZ 36711 (M, k); Tuxpan de Bolaños (21.874, -104.014), KU 112244 (m); Nayarit; Cucharas, Río Acaponeta (22.821, -105.306), USNM 509039–40 (m); 3 km (by road) E El Duraznito, 1,770 m (22.131, -104.728), LSUMZ 36836–38 (m); Ocota Airstrip, 1,900 m (21.85, -104.21), USNM 523469 (m); Navarrete, 300 ft. (21.648, -105.117), KU 111708 (m); Paso de Soquilpa, 8.8 mi. E San Blas (21.603, -105.183), USNM 509043 (m); (30) 2 km S La Cucaracha, 307 m (21.01, -105.14), LSUMZ 36641 (M, k); Sinaloa; 18 km NNE Choix (26.857, -108.236), KU 89259 (aM); 1.5 mi. ENE El Cajon, 3,700 ft. (26.819, -108.147), KU 100252 (aM); (16) 13 km SE Pericos, 85 m (25.015, -107.599), CNMA 44507 (M); 5 km SW [El] Palmito, 6,100 ft. (23.526, -105.869), KU 95031–32 (m); Chupaderos, 3 mi. SW Copala (23.371, -105.956), KU 105629 (m); 5 mi. NW Mazatlán (23.334, -106.462), KU 85744 (m); 3 mi. E El Roble (23.245, -106.160), KU 105611–12 (m); 7 km SE Concordia, 182 m (23.245, -106.025), LSUMZ 36634–35 (m); 8 km NW Villa...
Union (23.240, -106.276), KU 95952 (m); 7 mi. ENE Plomosas, 6,000 ft. (23.092, -105.404), KU 97145 (m); Rosario (21.603, -105.183), USNM 91394–95 (m)

*T. bottae* (M = 2)

**MEXICO:** *Sinaloa; (35)* 2 km E El Cajon de Cancio, 525 m (26.769, -108.214), LSUMZ 36755 (M); (36) Baroten, 4 km SW El Fuerte, 78 m (26.399, -108.659), LSUMZ 36630 (M)

*T. mazama* (M = 2)

**CALIFORNIA:** *Siskiyou Co.;* Antelope Creek, 1 mi. N Tennant, 4,700 ft. (41.597, -121.909), MVZ 171042 (M). **WASHINGTON:** *Mason Co.;* 2 mi. N Shelton on Hwy 101, Shelton Airport (47.2336, -123.1461), LSUMZ 34383 (M)

*T. talpoides* (M = 2)

**CALIFORNIA:** *Madera Co.;* Agnew Meadow, 9.5 mi. W Mammoth Lakes (37.683, -119.094), MVZ 176455 (M); **NEW MEXICO:** *Cibola Co.;* Mirabel Spring, 6.5 mi. S San Mateo (35.143, -107.640), LSUMZ 29581 (M)

*Orthogeomys hispidus* (M = 2)

**BELIZE:** *Cayo District;* 3 km W Belmopan (17.25, -88.79), LSUMZ 29232 (M). **MEXICO:** *Tamaulipas;* 19 km S, 9 km W Llera de Canales, 177 m (23.145, -99.115), LSUMZ 36767 (M)
APPENDIX 2.2
LIST OF PRIMERS AND THEIR ANNEALING TEMPERATURES

Primers used in this dissertation for both PCR and sequencing. $T_a = \text{PCR annealing temperature}$. Primers designated with an asterisk are internal primers used in cycle sequencing reactions only.

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GenBank numbers for sequences used in Chapter 2. Numbers 1–41 were used in full, multi-locus analyses, numbers 42–70 were used for Cytb analyses only, and numbers 71–72 are ancient DNA sequences. Other ancient DNA sequences that did not fit the minimum length requirement of GenBank are provided in Appendix 2.4. Loci that could not be successfully sequenced for an individual are indicated by N/A. GenBank sequences JX520323 – JX520599 and JX573116 are new to Chapter 2.

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APPENDIX 2.4
LIST OF ANCIENT DNA SEQUENCES USED IN CHAPTER 2

Four ancient DNA *Thomomys* cytochrome *b* sequences that did not fit the minimum length requirements for GenBank submission are shown here in nexus format, aligned to a reference *T. sheldoni* sequence (GenBank number HQ141717). CAS 11082 is *T. umbrinus* and USNM 523456, 523468, and 523465 are *T. sheldoni*. Locality information for these individuals is found in Appendix 2.2.

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USNM_523456
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GCCAA
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ATAGCTACTGCATTGTTGGGATA CGTATTACCC

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TAGTCATATCTGCCGAGACGTAAATTACGGGTGACTAATCC
GCTACATACATGCCAA
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GGCTCTTACCTCTATAAAGAAACATGGAACGTA GGCATCTGCTTTATTTCTTAACA
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USNM_523465

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USNM_523468

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HQ141717

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134
CATATTAAAACCGAATGATACCTTTTTATTGCTACGCTATTCTACGATCTATCCCTA
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APPENDIX 2.5
JOURNAL COPYRIGHT PERMISSION

Date: February 20, 2013

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"Resurrection and redescription of the pocket gopher Thomomys sheldonii from the Sierra Madre Occidental of Mexico" by Mathis, et al. anticipated in *Journal of Mammalogy* 94.3 (2013).

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Sincerely,

Lindsey Givens
Publishing Specialist
Allen Press Publishing Services

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We have elected not to use this material
APPENDIX 3.1
LIST OF SPECIMENS EXAMINED IN CHAPTER 3

Specimens new to this study are deposited in the Collection of Mammals, Louisiana State University Museum of Natural Science (LSUMZ) or the Colección Nacional de Mamíferos, Instituto de Biologia, Universidad Nacional Autónoma de México (CNMA). Other specimens used in this study are housed in the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), the United States National Museum of Natural History (USNM), the University of Kansas Natural History Museum (KU), and the California Academy of Sciences (CAS). Specimens used in the molecular analyses are designated “M” (“aM” for ancient DNA), those with chromosomal data “k”, those used in the morphometric analysis of the auditory meatus “audm”, and those used in the cranial morphometric analyses “m”. Sample sizes for each kind of analysis are indicated following the taxon names. Boldface numbers in parentheses before locality names refer to mapped localities in Fig. 3.1. Localities are listed north to south within states.

*T. nayarensis* (M = 6, aM = 2, k = 4, audm = 9, m = 9)

**MEXICO:** *Nayarit;* (9) 8.5 km N, 7 km W Mesa del Nayar (formerly listed by Hafner et al. [2011] as “22 km S, 3 km E Santa Teresa”), 2,200 m (22.29, -104.721), LSUMZ 36750 (M, audm), 36751–52 (M, audm, m), 36794 (M, k, audm), 36796 (M, k, audm, m); 36797 (audm, k); Mesa del Nayar, 4,500 ft. (22.197, -104.65), USNM 511560–62 (m), 511563–64 (aM, audm, m); (10) 1 km S Mesa del Nayar, 1,290 m (22.197, -104.65), LSUMZ 36830 (M, k, audm, m).

*T. s. sheldoni* (M = 4, aM = 3, audm = 9, k = 2, m = 30)

**MEXICO:** *Durango;* 22 mi. WSW Durango, 7,900 ft (23.92, -104.98), CAS 12277–78 (m); El Salto (23.779, -105.361), USNM 946111, 94647–49 (m); 33 km S, 7 km W Durango, 2,420 m (23.707, -104.732), LSUMZ 36811 (m); 43 km S, 32 km W Vincente Guerrero, 2,600 m (23.339,
-104.291), LSUMZ 36810 (m); **Nayarit; (5)** Santa Teresa, 6,800 ft. (22.488, -104.753), USNM 523456 (aM, audm, m), 90823 (m), 90826 (audm, m), 523467 (audm), 523468 (aM); (6) Santa Teresa, 13 km SW; Rancho Viejo (22.409, -104.835), USNM 523458–59 (m), 523463 (audm, m), 523464 (audm), 523465 (aM, audm, m), 523466 (audm); (7) 3 km (by road) SE Santa Gertrudis, 2,360 m (22.376, -104.811), LSUMZ 36831 (M, m), 36832 (audm), 36834 (audm, m), **Zacatecas;** 8 mi. W Milpillas, 60 mi. W Fresnillo, 8,300 ft. (23.07, -103.8), CAS 11083 (m); 8 mi. S Chalchuites (= Chalchihuites), 8,600 ft. (23.36, -103.88), CAS 13000, 13162–63 (m); (3) 6 km N, 15 km W Valparaiso, 2,730 m (22.827, -103.72), LSUMZ 36804 (M, m, k), 36805–06 (m); Valparaiso Mountains (22.827, -103.72), USNM 91985, 91987, 91994 (m); Sierra Madre (22.601, -104.333), USNM 90830–32 (m); (4) Santa Cruz, 2,480 m (22.41, -104.346), LSUMZ 36800 (M, k).

**T. umbrinus** (M = 4, aM = 1, k = 2)

**MEXICO: Durango; (1)** 1.5 mi. S (by road) Morcillo (23.732, -105.676), MVZ 150455 (M);

**Zacatecas; (2)** 7 km S, 8 km E Jiménez de Teul, 2,450 m (23.214, -103.737), LSUMZ 36713 (M); (14) 4 km N, 3.5 km W Monte Escobedo, 2,430 m (22.342, -103.599), LSUMZ 36801 (M, k); (15) 2.5 mi. N Moyahua, 4,400 ft. (21.3, -103.16), CAS 11082 (aM).

**T. atrovarius** (M = 3, aM = 1, k = 1, audm = 5, m = 22)

**MEXICO: Durango;** 1 mi. SW Revolcaderos (23.6, -105.85), USNM 375708 (m); **Jalisco; (13)** 6 km N, 12 km W Bolaños, 2,400 m (21.92, -103.893), LSUMZ 36711 (M, audm, k); Tuxpan de Bolaños (21.874, -104.014), KU 112244 (m); **Nayarit;** Cucharas, Río Acaponeta (22.821, -105.306), USNM 509039–40 (m); (8) 10 km E Acoponeta, 213 m (22.48, -105.25), LSUMZ 36636 (M); (11) 3 km (by road) E El Duraznito, 1,770 m (22.131, -104.728), LSUMZ 36836 (M, audm, m), 36835 (audm), 36837–38 (audm, m); (12) Ocota Airstrip, 1,900 m (21.85, -104.21),
USNM 523469 (aM, m); Navarrete, 300 ft. (21.648, -105.117), KU 111708 (m); Paso de Soquilpa, 8.8 mi. E San Blas (21.603, -105.183), USNM 509043 (m); Sinaloa; 5 km SW [El] Palmito, 6,100 ft. (23.526, -105.869), KU 95031–32 (m); Chupaderos, 3 mi. SW Copala (23.371, -105.956), KU 105629 (m); 5 mi. NW Mazatlán (23.334, -106.462), KU 85744 (m); 3 mi. E El Roble (23.245, -106.160), KU 105611–12 (m); 7 km SE Concordia, 182 m (23.245, -106.025), LSUMZ 36634–35 (m); 8 km NW Villa Union (23.240, -106.276), KU 95952 (m); 7 mi. ENE Plomosas, 6,000 ft. (23.092, -105.404), KU 97145 (m); Rosario (21.603, -105.183), USNM 91394–95 (m).

*T. bottae* (M = 2)

**MEXICO: Sinaloa;** (35) 2 km E El Cajon de Cancio, 525 m (26.769, -108.214), LSUMZ 36755 (M); (36) Baroten, 4 km SW El Fuerte, 78 m (26.399, -108.659), LSUMZ 36630 (M).

*T. mazama* (M = 2)

**CALIFORNIA: Siskiyou Co.;** Antelope Creek, 1 mi. N Tennant, 4,700 ft. (41.597, -121.909), MVZ 171042 (M). **WASHINGTON: Mason Co.;** 2 mi. N Shelton on Hwy 101, Shelton Airport (47.2336, -123.1461), LSUMZ 34383 (M).

*T. talpoides* (M = 2)

**CALIFORNIA: Madera Co.;** Agnew Meadow, 9.5 mi. W Mammoth Lakes (37.683, -119.094), MVZ 176455 (M); **NEW MEXICO: Cibola Co.;** Mirabel Spring, 6.5 mi. S San Mateo (35.143, -107.640), LSUMZ 29581 (M).

*Orthogeomys hispidus* (M = 2)

**BELIZE: Cayo District;** 3 km W Belmopan (17.25, -88.79), LSUMZ 29232 (M). **MEXICO: Tamaulipas;** 19 km S, 9 km W Llera de Canales, 177 m (23.145, -99.115), LSUMZ 36767 (M).
### APPENDIX 3.2
GENBANK SEQUENCES DEPOSITED FOR CHAPTER 3

GenBank numbers for sequences used in Chapter 3. Museum acronyms are listed in Appendix 3.1. Seven ancient DNA sequences that did not fit the minimum length requirement of GenBank are provided in Appendix 3.3. Loci that could not be sequenced successfully for an individual are indicated by N/A. GenBank sequences KC525216 – KC525244 are new to Chapter 3.

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APPENDIX 3.3
LIST OF ANCIENT DNA SEQUENCES USED IN CHAPTER 3

Seven ancient DNA *Thomomys* cytochrome *b* sequences that did not fit the minimum length requirements for GenBank submission are shown here in nexus format, aligned to a reference *T. sheldoni* sequence (GenBank number HQ141717). USNM 51163 and 51164 are *T. nayarensis*, USNM 523456, 523465, and 523468 are *T. sheldoni*, CAS 11082 is *T. umbrinus*, and USNM 523469 is *T. atrovarius*. Locality information for these individuals is found in Appendix 3.1.

USNM_511563

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USNM_511564

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USNM_523465

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143
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145
ATGACAATTATTCGCAAGTCKCATCCGCTATTTAAGATTGTAAACCACGCCTTCATT
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GAACCTTATGATACATCTTTTCTACATCAGAATGGAGCAACACCAAGTCGAAC
CACCATTATCATCATCAGGGCAAAACAGGCTCAATCTTTTGCTATTATATTATTAT
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APPENDIX 4.1
LIST OF SPECIMENS EXAMINED IN CHAPTER 4

Specimens new to this study are deposited in the Collection of Mammals, Louisiana State
University Museum of Natural Science (LSUMZ). Other specimens used in this study are housed
in the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), the United
States National Museum of Natural History (NMNH), the University of Kansas Natural History
Museum (KU), the Colección Nacional de Mamíferos, Instituto de Biología, Universidad
Nacional Autónoma de México (CNMA), the Museum of Southwestern Biology (MSB), the
California Academy of Sciences (CAS), and the New Mexico Museum of Natural History
(NMMNH). Specimens used in the molecular analyses are designated “M”, those used in the
chromosomal analysis “k”, those used in the morphometric analyses “m”, and those used in the
allozyme analysis designated “a”. Sample sizes for each type of analysis are indicated following
the taxon names. Boldface numbers in parentheses before locality names refer to mapped
localities in Figs. 4.1 and 4.2. Localities are listed north to south within states.

*T. u. intermedius* (M= 7, m = 56, k = 5, a =102)

ARIZONA: *Cochise County*; Fort Huachuca (31.563, -110.334), NMNH 33882 (m); *Pima
County*; Empire Ranch, E from Santa Rita Mountains (31.785, -110.642), NMNH 250581 (m);
Santa Rita Mountains, 36 mi. S Tucson, Florida Canyon, Santa Rita Range Reserve Headquarters
(31.774, -110.868), NMNH 272496 (m), Santa Rita Mountains, 42 mi. S Tucson, Lower Madera
(White House) Canyon (31.741, -110.941), NMNH 272483–86, 272489–90 (m); Santa Rita
Mountains, mouth of Madera Canyon, NMNH 262818 (m); Santa Rita Mountains, Stone Cabin
Canyon, NMNH 244076 (m); Santa Rita Mountains, 35 mi. S Tucson, Kimmerling (Old Parker)
Ranch, NMNH 272491, 272493 (m); *Santa Cruz County*; Santa Rita Mountains, Madera
Canyon (31.725, -110.881), NMNH 229472, 229475–76, 229478 (m); Yanks Tank, Yanks
Canyon, Parajito Mts., 1,250 m (31.425, -111.183), MVZ 170082–83 (m); Pajarito Mountains, Peña Blanca Spring (31.403, -111.087), NMNH 262825 (m); Tumacacori Mountains, Peña Blanca Spring, Peña Blanca Canyon (31.403, -111.087), NMNH 250586 (m); 4 mi. N, 9 mi. W Nogales, Peña Blanca Spring (31.389, -111.092), KU 22768–22772 (m); (1) Sycamore Canyon, Patagonia Mts., 1,341 m (31.386, -110.743), MVZ 148307 (M), MVZ 148306–18 (a); NEW MEXICO: Hidalgo County; Animas Mountains, Aspen Spring, T31S, R19W, Sec. 33, 7,300 ft. (31.567, -108.777), MSB 11059 (m); Animas Mountains, Mouth of Indian Creek Canyon (31.547, -108.717), MSB 25189 (m); Animas Mountains, Indian Creek Canyon (31.547, -108.717), MSB 25190 (m); Animas Mountains, Horse Thief Canyon, 0.25 mi. W Horse Thief, T32S, R19W, Sec. 34 (31.481, -108.766), MSB 45985 (m); (2) Animas Mountains, 5.2 mi. N, 8.7 mi. W Hilo Peak (31.472, -108.747), NMMNH 1920 (M); 12 mi. W Antelope Wells, mouth of Whitewater Canyon, T34S, R19W (31.35, -108.703), MSB 8234 (m); 11.5 mi. W Antelope Wells, mouth of Whitewater Canyon, T34S, R19W, Sec. 13 (31.35, -108.703), MSB 2204 (m); MEXICO: Chihuahua; Río El Gavilán, 7 mi. SW Pacheco (30.015, -108.417), MVZ 109657–58, 109661–62, 109664, 109668, 109670 (m); near Colonia Garcia (=10 mi. NE Colonia Garcia, Pilares Canyon; Anderson 1972) (30.08, -108.21), NMNH 98204–05, 98208 (m); (4) 2.4 mi. NE (by road) Colonia Garcia (30.002, -108.32), MVZ 150606 (M), MVZ 150585–610 (a); (5) 2 km S, 0.5 km E Colonia Garcia, 2,200 m (29.958, -108.333), LSUMZ 36721 (M, k); (6) 6 km E Colonia Garcia, 2,200 m (29.974, -108.275), LSUMZ 36728 (M, k), LSUMZ 36727, 26729–30 (m, k); Sonora; near Mina San Eufracio, 10 mi. NE Chinapa (30.526, -109.921), MVZ 75003–04 (m); Chinapas, 10 mi. east, Sonora River Valley (30.45, -109.865), NMNH 250893 (m); (3) 1 mi. S Moctezuma (29.802, -109.667), MVZ 147094–96 (m); MVZ 147097 (M, m), MVZ 147085–101, 148869–75 (a); ca. 1 mi. S (by road) Moctezuma (29.802, -109.667), MVZ 148871
(m); Moctezuma (29.79, -109.69), MVZ 74949, 74951 (m); (8) 30 km SW Moctezuma, 1,000 m
(29.4, -109.5), MSB 61113 (M); E bank Río Yaqui at El Novillo (28.98, -109.629), MVZ
148891–93, 148895, 148897 (m), MVZ 148888–99 (a); W bank of Río Yaqui at El Novillo
(28.98, -109.63), MVZ 148900–08 (a); Bacanora (28.979, -109.398), MVZ 148876, 148880,
148882–85 (m), MVZ 148876–87 (a); ca. 1 mi. N (by road) Sahuaripa (29.07, -109.24), MVZ
148909–14 (a).

_T. u. goldmani_ (M= 15, m = 17, k = 18, a = 35)

**MEXICO: Chihuahua;** Cañón del Alamo, Sierra del Nido, 7,000 ft. (29.485, -106.77), MVZ
124839, 124842 (m); (10) Cañón del Arroyo Santa Clara, Sierra del Nido (29.366, -106.572),
MVZ 147083 (M, m, a), MVZ 147084 (a); Arroyo el Mesteño, Sierra del Nido, 7,800 ft. (29.392,
-106.899), MVZ 128279–80 (m); (9) 9 km N Santo Tomas, 2,100 m (28.731, -107.648), LSUMZ
36694 (M, m); (11) 8.4 mi. W (by road) Cuauhtémoc (28.387, -107.006), MVZ 150505–07 (m),
MVZ 150508 (M, m), MVZ 150491–509 (a); (12) 10 km N, 5 km E Meoqui, 1,160 m (28.316, -
105.431), LSUMZ 36719 (M, m, k); 1 mi. NW Camargo (27.68, -105.16), KU 34296 (m); 1 mi.
S Camargo (27.65, -105.17), KU 55557–58 (m); (14) 5 km S Ciudad Camargo, 1,280 m (27.628,
-105.121), LSUMZ 36717 (m, k), LSUMZ 36718 (M, m, k); 1.5 mi. N Boquilla de Conchos, 14
mi. SW Ciudad Camargo (27.565, -105.4), MVZ 122977 (m); Jimenez (27.12, -104.95), KU
66128 (m); El Rosario (26.87, -105.14), KU 73641–42 (m); 10 mi. SE of Parral (26.847, -
105.55), KU 66130 (m); (15) Rio Belleza, 20 km N, 17 km E El Vergel, 1,730 m (26.655, -
106.22), LSUMZ 36745 (m, k), LSUMZ 36747 (M, m, k); _Coahuila; (24) _15 km (by road) NW
La Flor de Jimulco (at km 11), 1,230 m (25.225, -103.448), LSUMZ 36602 (M); LSUMZ
36590–91 (m); _Durango; (16) _1 km SE El Ojito, 2,250 m (26.731, -106.043), LSUMZ 36701
(M, m, k), LSUMZ 36702 (m, k); (17) 6 km S, 13 km W El Ojito, 1,770 m (26.683, -106.186),
LSUMZ 36741 (M, k); 13 km S, 15 km E El Ojito (26.615, -105.864), LSUMZ 36703 (m, k); 20 km S, 22 km E El Ojito, 1,900 m (26.542, -105.785), LSUMZ 36704 (m, k); (18) 14.7 mi. N (by road) Las Nieves (26.537, -105.492), MVZ 150470 (M), MVZ 150461–74 (a); (19) 10 km N, 20 km W Ocampo, 1,800 m (26.535, -105.711), LSUMZ 36705 (M, k); LSUMZ 36706–08 (m, k); (20) 3 km W Ocampo, 1,750 m (26.459, -105.543), LSUMZ 36709 (M, k), LSUMZ 36710 (m, k); (21) 50 km N, 20 km W Bermejillo, 1,140 m (26.34, -103.803), LSUMZ 34350 (m), LSUMZ 34351 (M, m); Mapimí (25.831, -103.842), NMNH 58076 (m); 1 mi. WSW Mapimí (25.81, -103.86), KU 40216 (m); (22) 2 km S, 8 km E El Palmito, 1,500 m (25.597, -104.925), LSUMZ 36812 (M, k); (23) Rio Nazas, 2 km S, 29 km E Rodeo, 1,277 m (25.157, -104.266), LSUMZ 36813 (M, k).

_T. u. durangi_ (M = 3, m = 11, k = 1, a = 30)

**MEXICO: Durango; (29)** 1.5 mi. S (by road) Morcillo (23.732, -105.676), MVZ 150455 (M, m), MVZ 150457, 150460 (m), MVZ 150448–60 (a); Durango (24.03, -104.67), NMNH 94605, 94607 (m); (28) La Boca del Mezquital, 1,900 m (23.774, -104.445), LSUMZ 36807 (M, m, k), LSUMZ 36808–09 (m, k); 3 mi. E Las Adjuntas (23.82, -104.2), KU 67619 (m); **Zacatecas; (30)** 10 km S, 2 km W Sombrerete, 2,130 m (23.617, -103.73), MVZ 153758 (M), MVZ 153754, 153756–57 (m), MVZ 153746–62 (a).

_T. u. umbrinus_ (M = 12, m = 99, k = 5, a = 121)

**MEXICO: Guanajuato;** Santa Rosa (21.068, -101.202), NMNH 81680, 81683–86, 81688 (m); **Hidalgo;** El Chico, Sierra De Pachuca (20.22, -98.73), NMNH 51886, 51888 (m); Real del Monte (20.13, -98.67), NMNH 26356–57 (m); Tulancingo (20.08, -98.37), NMNH 55624 (m); **Mexico; (42)** 34 rd km E Zitácuaro, (Bosencheve) (19.416, -100.124), LSUMZ 25101 (M, m), LSUMZ 25103 (m); Salazar (19.3, -99.42), NMNH 50119, 50123, 50127–31, 50133 (m); 10 km
S, 16 km W Toluca, 3,000 m (19.20, -99.815), LSUMZ 36128 (m), MVZ 152829–49 (a); Volcán Toluca, N Slope (19.143, -99.757), NMNH 55912–14, 55916, 55918 (m); Nevado De Toluca, 4 mi. S Raices (19.101, -99.804), NMNH 329719, 329723 (m); Nevado De Toluca, 16 mi. SSW Toluca (19.073, -99.7615), NMNH 329709–10 (m); (43) 25 km N Valle de Bravo, 2,438 m (19.422, -100.129), LSUMZ 36074 (M); (45) 5.5 km S, 13 km E Amecameca de Juárez (19.08, -98.63), MVZ 153866 (M), MVZ 153850–67 (a); (44) Volcán Iztaccíhuatl, 4 km N Paso de Cortez, 3,842 m (19.064, -98.383), CNMA 42505 (M); Mt. Popocatépetl (19.024, -98.6251), NMNH 51885 (m); Michoacán; 4 mi. S Pátzcuaro, 7,800 ft. (19.45, -101.609), MVZ 100140 (m); 5 mi. S Pátzcuaro, 7,800 ft. (19.443, -101.609), MVZ 100150 (m); (41) 6.5 km S Pátzcuaro, 2,200 m (19.421, -101.609), LSUMZ 34359 (M), MVZ 153825–27 (m), MVZ 153812–828 (a); 10 km SE Pátzcuaro, Cerro del Burro (19.45, -101.54), LSUMZ 25100 (m); Morelos; ca. 8 km SW Parres (in D.F.), 3,005 m (19.094, -99.214), LSUMZ 36760 (m); Puebla; San Martín Texmelucan (19.28, -98.43), NMNH 55622 (m); San Martín (19.28, -98.43), NMNH 55623 (m); Malinche Volcano, S Slope (19.23, -98.03), NMNH 540988 (m); Mount Orizaba (19.035, -97.23), NMNH 53605, 53607, 53613–16, 53659–60 (m); (46) Boca del Monte, 3.5 km S and 3 km E Esperanza, 2,450 m (18.83, -97.328), MVZ 153877 (M), MVZ 153868–84 (a); Hacienda San Pedro Coxtocan, km 96.5 on Puebla-Mexico Highway, NMNH 540967–68, 540981 (m); San Luis Potosi; Palma, 7 km NW (22.75, -101.83), NMNH 296785 (m); 7 km NW Palma (22.75, -101.83), LSUMZ 4191 (m); Cerro Peñón Blanco (22.519, -101.676), LSUMZ 4196 (m); 1 km N Arenal (22.18, -100.97), LSUMZ 5051–52 (m); 1 km S Arenal (22.16, -100.97), LSUMZ 5045 (m); La Tinaja (22.36, -100.85), NMNH 82059–62, 82064 (m); (40) Ventura (22.26, -100.88), MVZ 153799 (M), MVZ 153788–809 (a), LSUMZ 5027, 5034 (m); 3 km SW San Isidro (22.064, -100.671), LSUMZ 5050, 5061, 5067 (m); Alvarez (22.05, -100.617),
NMNH 266331 (m); 6 km S San Isidro (22.029, -100.65), LSUMZ 5054–57 (m); 4 mi. E Villa de Arriaga (on Hwy. 80) (21.946, -101.332), MVZ 139813, 139815 (m); (39) 11 km N, 12 km E Arriaga, 2,030 m (21.90, -101.266), MVZ 153810 (M, a), MVZ 153811 (m, a); 1 km S Arriaga (21.891, -101.383), LSUMZ 5074, 5080–81 (m); **Tlaxcala;** 6 km N Pico de Volcán la Malinche (19.28, -98.04), LSUMZ 36373 (m); **Veracruz;** Boca del Monte (21.12, -97.57), NMNH 64094, 64096–97 (m); **Zacatecas;** (31) 7 km S, 8 km E Jiménez de Teul, 2,450 m (23.214, -103.737), LSUMZ 36713 (M, k), LSUMZ 36714 (m, k); (36) 3 km N Ojocaliente, 2,030 m (22.597, -102.251), MVZ 153778 (M), MVZ 153764–87 (a); Berriozabal (22.55, -102.32), NMNH 57973, 79502 (m); (35) 4 km N, 3.5 km W Monte Escobedo, 2,430 m (22.342, -103.599), LSUMZ 36801 (M, k), LSUMZ 36802 (m, k), LSUMZ 36803 (m); 3 mi. NW Monte Escobedo (22.33, -103.617), KU 107548–52, 107554, 107556 (m); Plateado (21.95, -103.1), NMNH 90837 (m); (38) 5 km S, 18 km E Jalpa, 2,550 m (21.605, -102.836), LSUMZ 36712 (M, m, k); 2.5 mi. N Moyahua, 4,400 ft. (21.3, -103.16), CAS 11082 (m).

*T. nayarensis* (M = 2, k = 1)

**MEXICO: Nayarit;** (32) 22 km S, 3 km E Santa Teresa, 2,200 m (22.29, -104.721), LSUMZ 36750 (M), (33) 1 km S Mesa del Nayar, 1,290 m (22.197, -104.65), LSUMZ 36830 (M, k).

*T. sheldoni* (M = 3, k = 3)

**MEXICO: Chihuahua;** (7) 4 km S, 1 km E Colonia Garcia, 2,200 m (29.937, -108.327), LSUMZ 36723 (M, k); (13) 5 km SE Creel, 2,033 m (27.714, -107.608), LSUMZ 36696 (M, k);

**Durango;** (27) 12 km E El Salto, 2,490 m (23.783, -105.239), LSUMZ 34354 (M), (26) 1 mi. E La Ciudad, 2,590 m (23.732, -105.676), MVZ 150444 (M, k).

*T. atrovarius* (M = 3)
MEXICO: Jalisco; (34) 6 km N, 12 km W Bolaños, 2,400 m (21.92, -103.893), LSUMZ 36711 (M); Nayarit; (37) 2 km S La Cucaracha, 307 m (21.01, -105.14), LSUMZ 36641 (M); Sinaloa; (25) 13 km SE Pericos, 85 m (25.015, -107.599), CNMA 44507 (M).

*T. bottae* (M = 2)

MEXICO: Sinaloa; 2 km E El Cajon de Cancio, 525 m (26.769, -108.214), LSUMZ 36755 (M); Baroten, 4 km SW El Fuerte, 78 m (26.399, -108.659), LSUMZ 36630 (M).

*T. mazama* (M = 2)

CALIFORNIA: Siskiyou Co.; Antelope Creek, 1 mi. N Tennant, 4,700 ft. (41.597, -121.909), MVZ 171042 (M). WASHINGTON: Mason Co.; 2 mi. N Shelton on Hwy 101, Shelton Airport (47.2336, -123.1461), LSUMZ 34383 (M).

*T. talpoides* (M = 2)

CALIFORNIA: Madera Co.; Agnew Meadow, 9.5 mi. W Mammoth Lakes (37.683, -119.094), MVZ 176455 (M); NEW MEXICO: Cibola Co.; Mirabel Spring, 6.5 mi. S San Mateo (35.143, -107.640), LSUMZ 29581 (M).

*Orthogeomys hispidus* (M = 2)

BELIZE: Cayo District; 3 km W Belmopan (17.25, -88.79), LSUMZ 29232 (M). MEXICO: Tamaulipas; 19 km S, 9 km W Llera de Canales, 177 m (23.145, -99.115), LSUMZ 36767 (M).
APPENDIX 4.2
GENBANK SEQUENCES DEPOSITED FOR CHAPTER 4

GenBank numbers for sequences used in Chapter 4. Loci that were not sequenced for an individual are indicated by N/A. GenBank sequences KC589028 – KC589103 were newly generated for Chapter 4.

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VITA

Verity Lynn Mathis was born in Dayton, Tennessee to Karen Jean Mathis (nee Shapiro) and Jeffery Lee Mathis. She grew up in Grimesland, North Carolina where she attended G. R. Whitfield Elementary School and D. H. Conley High School. She graduated from North Carolina State University in 2000 with a B.S. degree in Zoology and a B.S. degree in Fisheries and Wildlife Sciences (wildlife concentration). While completing her bachelor’s degrees, she began volunteering in the mammal research and collections section of the North Carolina Museum of Natural Sciences. This experience, coupled with a love of mammalogy and wildlife science classes, set her on the path to future work in mammalogy and museum based science.

In January 2003 she joined the graduate program in the Department of Fishery and Wildlife Sciences at New Mexico State University, in the lab of Dr. Gary Roemer. She graduated with her M.S. degree in 2006. Her thesis involved the study of the role inbreeding avoidance plays in altering polygamy in banner-tailed kangaroo rats. In August 2006 she entered the Graduate School at Louisiana State University, working under the advisement of Dr. Mark Hafner in the Department of Biological Sciences and the Louisiana State University Museum of Natural Science. Before completing her dissertation, she accepted a job in December 2012 as a Conservation Resources Coordinator for the Mississippi Museum of Natural Science in Jackson, Mississippi, where she is a coordinator for the research and collections program.