Thērya

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La portada

Pocket gopher (*Megascapheus nigricans*): This genus of gophers is found from the southern part of Canada to the Trans-Mexican Volcanic Belt. The different species have colonized practically all habitats in these areas, and in several places, they are considered pests. The pocket gophers of the genus *Megascapheus* belong to the tribe Thomomyini, which to date is a group with several taxonomic complications (photo Ticul Álvarez).

Nuestro logo "Ozomatli"

El nombre de "Ozomatli" proviene del náhuatl se refiere al símbolo astrológico del mono en el calendario azteca, así como al dios de la danza y del fuego. Se relaciona con la alegría, la danza, el canto, las habilidades. Al signo decimoprimero en la cosmogonía mexica. "Ozomatli" es una representación pictórica de los mono arañas (*Ateles geoffroyi*). La especie de primate de más amplia distribución en México. " Es habitante de los bosques, sobre todo de los que están por donde sale el sol en Anáhuac. Tiene el dorso pequeño, es barrigudo y su cola, que a veces se enrosca, es larga. Sus manos y sus pies parecen de hombre; también sus uñas. Los Ozomatin gritan y silban y hacen visajes a la gente. Arrojan piedras y palos. Su cara es casi como la de una persona, pero tienen mucho pelo."

Contenido

Therya

Volumen 16, número 1 enero 2025

fascículo 46 https://mastozoologiamexicana.com/therya/index.php/THERYA/issue/view/49

Therya

El objetivo y la intención de *THERYA* es ser una revista científica para la publicación de artículos sobre los mamíferos. Estudios de investigación original, editoriales y artículos de revisión son bienvenidas.

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Editorial

Keep running your traplines: Celebrating the contributions of Jim and Carol Patton to the science and people of mammalogy

It is with pleasure that we introduce this volume, which honors the many contributions that Jim and Carol Patton have made to the discipline of mammalogy. Their influence extends well beyond the research they have conducted together and includes the profound impact they have had on the careers of researchers from around the world. Although the papers included here represent only a small subset of the students, postdoctoral scholars, and long-term collaborators who have worked with Jim and Carol, the list of contributors and the institutions they represent speak directly to the international scope of the Pattons' influence on the study of mammalian biology. The topics and taxa represented are equally broad and underscore the extent of the Pattons' expertise and the degree to which their mentorship has transcended academic boundaries.

While it was Jim who held a faculty position at U.C. Berkeley and who served and continues to serve as a curator in the Museum of Vertebrate Zoology, he has done it all in partnership with Carol. This includes joint field trips throughout the world, during which Carol worked side by side with Jim to set traps, prepare specimens, and explore mammalian diversity. Carol has also been a fundamental contributor to mentoring students and postdoctoral scholars and to organizing field trips for Berkeley's undergraduate class in mammalogy. Together, Jim and Carol have attended the annual meetings of the American Society of Mammalogists for more than 50 years, including (reportedly) while on their honeymoon. In short, it simply is not possible to celebrate Jim's career without also celebrating Carol and thus this volume is dedicated to both of them.

Gophers, genetics, and the Juruá: the pre-retirement era. Jim joined the faculty at U.C. Berkeley in 1969 (Figure 1). Over the next 30 years, Jim redefined our understanding of mammalian evolution by linking patterns of diversity and evolutionary relationships to organismal ecology in ways that had not been done previously. Central themes

Figure 1. Jim Patton writing field notes in Peru while wearing local, traditional face markings. Photo taken in 1968 just prior to Jim beginning his faculty position at U.C. Berkeley. Photo: Museum of Vertebrate Zoology Archives.

to his research included: 1) the causes and consequences of chromosomal evolution; 2) mechanisms underlying species boundaries in pocket gophers; 3) systematics and biogeography of North and South American rodents; and 4) interactions between ecology and landscape history in shaping the biodiversity of Amazonia. Upon his retirement from faculty responsibilities in 2001, many people gathered at the Museum of Vertebrate Zoology for "Pattonfest," a multi-day celebration of Jim's extraordinary career. The edited volume that grew out of Pattonfest (Lacey and Myers 2005) reflects the conceptual, geographic, and taxonomic breadth of Jim's research career. Notably, the volume contains a comprehensive review of Jim's personal and professional journey prior to 2001 (Robles and Greene 2005) that concludes with his reflections upon retirement, including the statement "I don't know what I will be doing ten years from now."

Grinnell revisited: the post-retirement era. Fast-forward twenty-five years, and Jim and Carol are still running their traplines! Since Jim's retirement, they have continued to document the diversity of western landscapes (Figures 2 and 3) and other regions of the world, and to connect with the people in those places. Their work has continued to expand our understanding of mammalian diversity, ecology and evolution, and to inspire new generations of biologists.

During the first decade of retirement, Jim and Carol spent much of each year in the Sierra Nevada Mountains as part of the Grinnell Resurvey Project. Conceived of by former MVZ Director Craig Moritz, Jim and Carol provided the essential "boots on the ground" effort needed to resurvey the mammals of Yosemite, Lassen, and Sequoia Kings Canyon National Parks a century after Joseph Grinnell and colleagues first characterized the vertebrates of these areas. In addition to generating critical insights into mammalian response to changing conditions, this project effectively launched a new discipline of museum-based research: temporal resurveys of faunal change.

Figure 2. Jim and Carol Patton at Whitney Well, Kelso Valley, California in 2018. Jim is preparing specimens in his field lab while Carol is keying out desert flowers. Photo: Marjorie Matocq.

Figure 3. Jim and Carol Patton checking traps near Shoshone, California in December 2025. Photo: Patrick Kelly.

More generally, the post-retirement era has seen a return to Jim's Grinnellian roots. He has undertaken significant taxonomic and phylogenetic revisions of several important lineages of California rodents, notably woodrats (genus *Neotoma*) and pocket mice (genus *Chaetodipus*). At the core of these projects are extensive field surveys of western North America (Figures 2 and 3) – now in their 80's, Jim and Carol still spend a part of each spring and fall trapping rodents. The resulting specimens – prepared with typical Patton perfection (Figure 4) – are being used in numerous ongoing studies of mammalian biology. In all aspects of their work, the Pattons embody the principles, practices, and dedication to natural history espoused by Grinnell (Figure 5).

To put things in more quantitative terms, since his retirement Jim has published more than 100 papers, book chapters, and monographs, as well as a seminal edited volume (Patton *et al.* 2020) on the mammals of South America for which he also co-authored many chapters. During the same period, he has contributed 11,500 specimens to the MVZ, with a lifetime total of more than 30,000 specimens and counting. Although he no longer teaches undergraduate classes, he has co-authored *A Manual of the Mammalia: An Homage to Lawlor's "Handbook of the Orders and Families of Living Mammals"* (Kelt and Patton 2020) a comprehensive, specimen-based lab manual for mammalogy based upon his three decades of teaching this course at Berkeley. This invaluable resource is increasingly viewed as *the* standard reference for teaching mammalogy courses. Jim's legacy as a teacher and mentor continues through two graduate

Figure 4. Jim preparing a specimen in the field in September 2011. Toiyabe Range, Nevada. Photo: Rebecca Rowe.

student awards given annually by the American Society of Mammalogists: the James L. Patton Award and the Carol and Jim Patton Award.

Shortly after retirement, Jim emptied out his faculty office, moving his microscopes, field notes, and most essential books to a nook located within the MVZ collections, where he continues to work while surrounded by specimens. Jim meets regularly with colleagues, museum visitors, and Berkeley students, resulting in a steady stream of foot traffic to his retirement office. Jim continues to generously share his time and encyclopedic knowledge with everyone who comes to him, providing keen insight and stimulating ideas, all while referring to himself as "just a rat trapper".

Honoring the Pattons. The idea for this volume originated with Sergio Ticul Álvarez-Castañeda who, as Editor-in-Chief of *Therya*, initiated the practice of organizing periodic collections of papers in honor of esteemed mammalogists. Ticul recruited Marjorie who then recruited Eileen to help bring the project to fruition. We solicited contributions from some of Jim's past students, postdocs, and long-time collaborators – collectively, these individuals span the full duration of Jim's career. The volume begins with two papers that highlight the continued importance of some of Jim's most profound contributions to the study of mammalian evolution: the role of chromosomal changes in speciation (Moritz and Potter) and the need for multi-dimensional assessments of species boundaries (Nachman). Jim is widely recognized for his use of emerging genetic technologies to study the evolution of mammalian diversity. The next three papers in the volume highlight the application of such approaches to the discovery of cryptic species (Geise *et al.*), to the identification of evolutionary relationships (Lessa and Parada), and to the evaluation of taxonomic units (Álvarez-Castañeda and Segura-Trujillo). The next two papers explore the mechanisms underlying species boundaries by examining interspecific hybridization in tuco-tucos (de Freitas and Ximines) and woodrats (Matocq *et al.*). As evidence of the broad reach of Jim's research, the following three papers highlight the role of small mammals in community ecology including predator-prey dynamics (Kelt *et al.*), host-parasite interactions (Adams *et al.*), and as indicators of ecosystem change over time (Stegner and Hadly). Finally, representing Jim's contributions to mammalian conservation, the last set of papers focus on the threats native mammal populations face in rapidly changing landscapes (Cypher *et al.*) and the critical importance of faunal surveys for monitoring changes in mammalian abundance and diversity over time (Yu *et al.* and Rickart *et al.*).

Figure 5. Jim greeting a recently captured woodrat near Shoshone, California in December 2025. This familiarity with organisms and their natural environments is the most fundamental component of evolutionary studies. Photo: Patrick Kelly.

Although no single volume can capture the full scope of the Pattons' impact on mammalogy, our hope is that this compilation of papers will bring renewed attention to a truly remarkable partnership that continues to generate new knowledge, promote forward-looking research on mammals, and inspire new generations of biologists (Figure 6). Many of the papers included here contain heartfelt statements from the authors regarding the ways in which their careers were shaped by Jim and Carol – sentiments that are broadly shared among those who have been influenced by their mentorship and friendship. As two of the many individuals who have benefitted from their advice and encouragement throughout our careers, we wish to express our sincere thanks to Jim and Carol for everything they have done over the years to support the science and people of mammalogy.

Figure 6. Jim and Carol Patton near Hackberry, Arizona in May 2018. Photo: Duke Rogers.

Acknowledgments

First, thank you to Sergio Ticul Álvarez-Castañeda for initiating this project and to Cintya A. Segura-Trujillo for her support in creating this volume. In addition to the authors themselves, we thank all those who shared their perspectives on Jim and Carol, as well as the many individuals who supported this project and conveyed their appreciation of Jim and Carol by providing expert review of these manuscripts. Thanks to: Kayce Bell, Jessica Blois, Robert Bradley, Chris Conroy, Chris Feldman, Harry Greene, Larry Heaney, Andrew Hope, Fabian Jaksic, Matina Kalcounis-Rüeppel, Jessica Light, Verity Mathis, Phil Myers, George Oliver, Bruce Patterson, Javier Rodríguez-Robles, Duke Rogers, Becca Rowe, Bob Timm, and Nate Upham. Preventing the Pattons from learning about this project until its release was a community effort; the authors are thrilled to finally be able to share these papers with Jim and Carol and look forward to the ensuing discussions!

Marjorie Matocq[1], and Eileen Lacey[2]

[1] Natural Resources and Environmental Science, University of Nevada at Reno. 1664 N. Virginia Street, Reno, NV 89557. USA. E-mail: mmatocq@unr.edu (MM).

[2] Museum of Vertebrate Zoology. University of California. 3101 Valley Life Sciences Building. Berkeley, CA 94720. USA. E-mail: ealacey@berkeley.edu (EL).

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Chromosome evolution and speciation: Revisiting Bush *et al*. (1977)

Craig Moritz[1*], and Sally Potter[2]

[1] Research School of Biology, The Australian National University, ACT 2601, Australia. Email: craig.moritz@anu.edu.au (CM).
[2] School of Natural Sciences, Macquarie University, Macquarie Park, NSW 2901, Australia. Email: sally.potter@mq.edu.au (SP).
* Corresponding author: https://orcid.org/0000-0001-5313-7279.

Whether and how chromosome change can drive speciation has long attracted the interest of evolutionary biologists. In a seminal paper, Bush *et al.* (1977) demonstrated wide variation in rates of macroscopic chromosome change across vertebrates and a positive correlation with rates of speciation at genus-level, and then considered possible causal processes. We revisit the key findings of this paper, highlighting how rapidly advancing knowledge of genome organisation and function shed new light on this long standing question. The central findings of Bush *et al.* (1977) have endured. There is now great opportunity to apply new genome technologies to understand the interaction of genome change and function in the classic systems of chromosomal speciation discovered through classical cytogenetics.

El hecho de si el cambio cromosómico puede impulsar la especiación y cómo puede hacerlo ha atraído desde hace tiempo el interés de los biólogos evolutivos. En un artículo fundamental, Bush *et al.* (1977) demostraron una amplia variación en las tasas de cambio cromosómico macroscópico en vertebrados y una correlación positiva con las tasas de especiación a nivel de género, y luego consideraron los posibles procesos causales. Repasamos los hallazgos clave de este artículo, destacando cómo el rápido avance del conocimiento de la organización y función del genoma arroja nueva luz sobre esta antigua pregunta. Los hallazgos centrales de Bush *et al.* (1977) han perdurado. Ahora existe una gran oportunidad de aplicar nuevas tecnologías genómicas para comprender la interacción del cambio y la función del genoma en los sistemas clásicos de especiación cromosómica descubiertos mediante la citogenética clásica.

Keywords: Chromosomal speciation; genome evolution; cytogenetics.

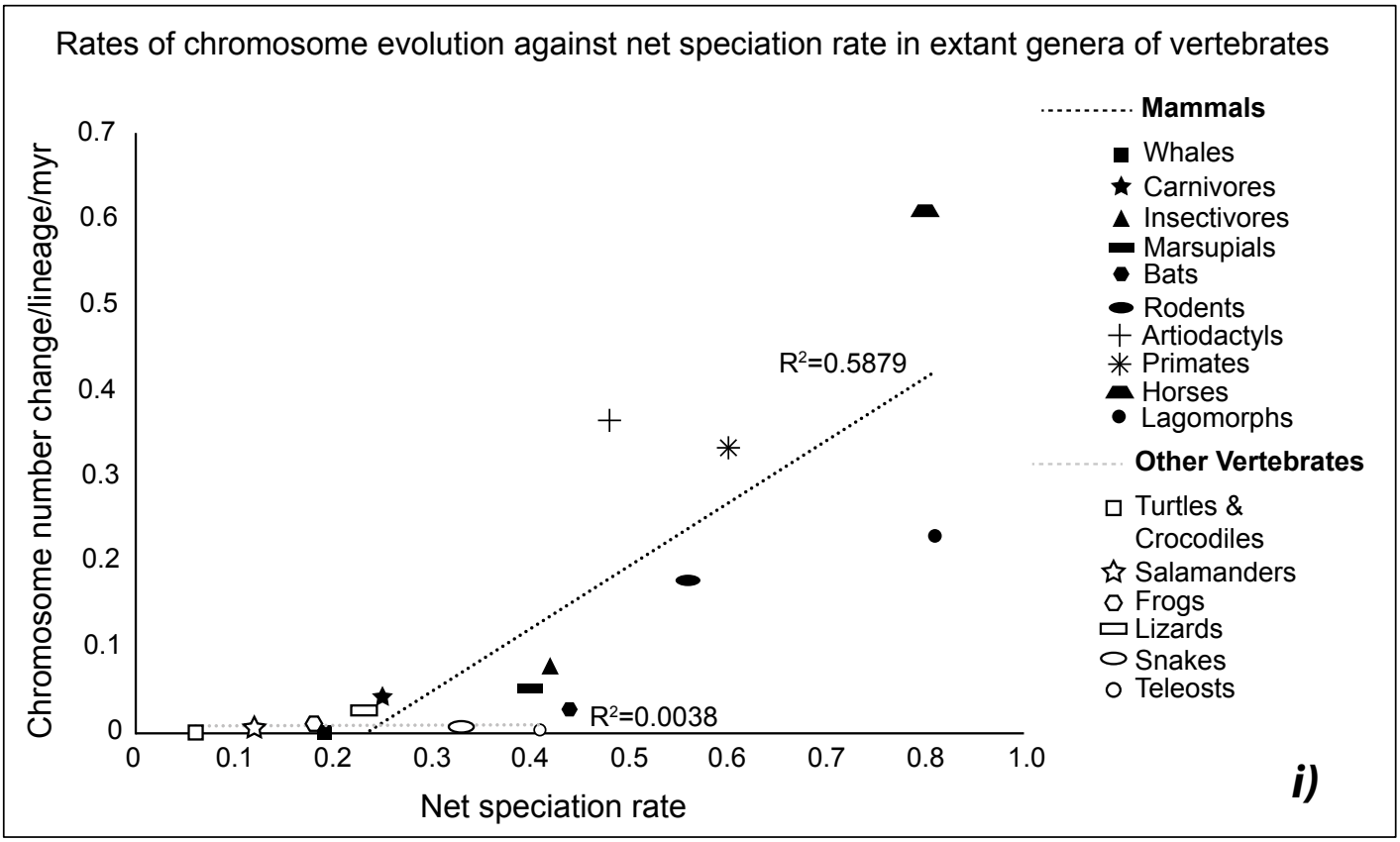
Introduction

Jim Patton, chromosomes and speciation. Jim Patton is an exemplary scholar and educator of all things relating to diversity of mammals. He is also a selfless mentor to early career researchers, including the lead author from the outset of his PhD, throughout his postdoctoral studies, and then as a faculty colleague. His seminal contributions are many and varied, including works on systematics, adaptation and speciation with a special focus on rodents, the most speciose order of mammals. His earliest works demonstrated the extraordinary diversity of chromosome number and form among rodents (*e. g*. Patton 1967) leading to many papers exploring how genome reorganisation contributed to speciation. Here we focus on one highly influential paper, Bush *et al.* (1977), on which Patton was senior author. We first summarise the context and key points of this paper. Then, some 47 years on, we revisit the key findings from the perspectives of new evidence on genome restructuring in mammals and also new theory.

The era of "classical cytogenetics" revealed substantial variation in chromosome organisation –differences in number of entire chromosomes and of chromosome arms– among closely related species. This, together with observations of disrupted meiosis in hybrids, quite naturally led to the conclusion that chromosome change could directly cause speciation (summarised in White 1978). At the same time, there was growing acceptance that genetic drift in small populations increases the likelihood of speciation – Wright's (1931, 1940) Shifting Balance Theory.

In this context, Bush *et al.* (1977) set out to test the prediction that rates of speciation across representative genera of vertebrates should be correlated with rates of chromosome change. The authors then linked differences in rates to ecological attributes that should lead to strong genetic drift. The key results (Figure1) were that: i) rates of both speciation and chromosome change are higher in mammals than in other classes of vertebrates; ii) across vertebrate genera, there is a strong correlation between these rates; and iii) mammalian genera with high rates of chromosome change do have ecological characteristics that can be expected to enhance drift. This quantitative analysis, together with a parallel finding for plants (Levin and Wilson 1976) was a seminal contribution to the developing theory of speciation by chromosome change. Bush *et al.* (1977) noted that correlation does not prove that chromosome changes can directly cause speciation, but they did point out several ways by which this might happen, including reduced fitness of hybrids, changes in gene regulation and creation of novel linkage groups.

In subsequent papers (Patton and Sherwood 1983; Patton 2004), Patton was less enamoured with the hypothesis that meiotic dysfunction was the link between chromosome change and speciation due to the dependence on



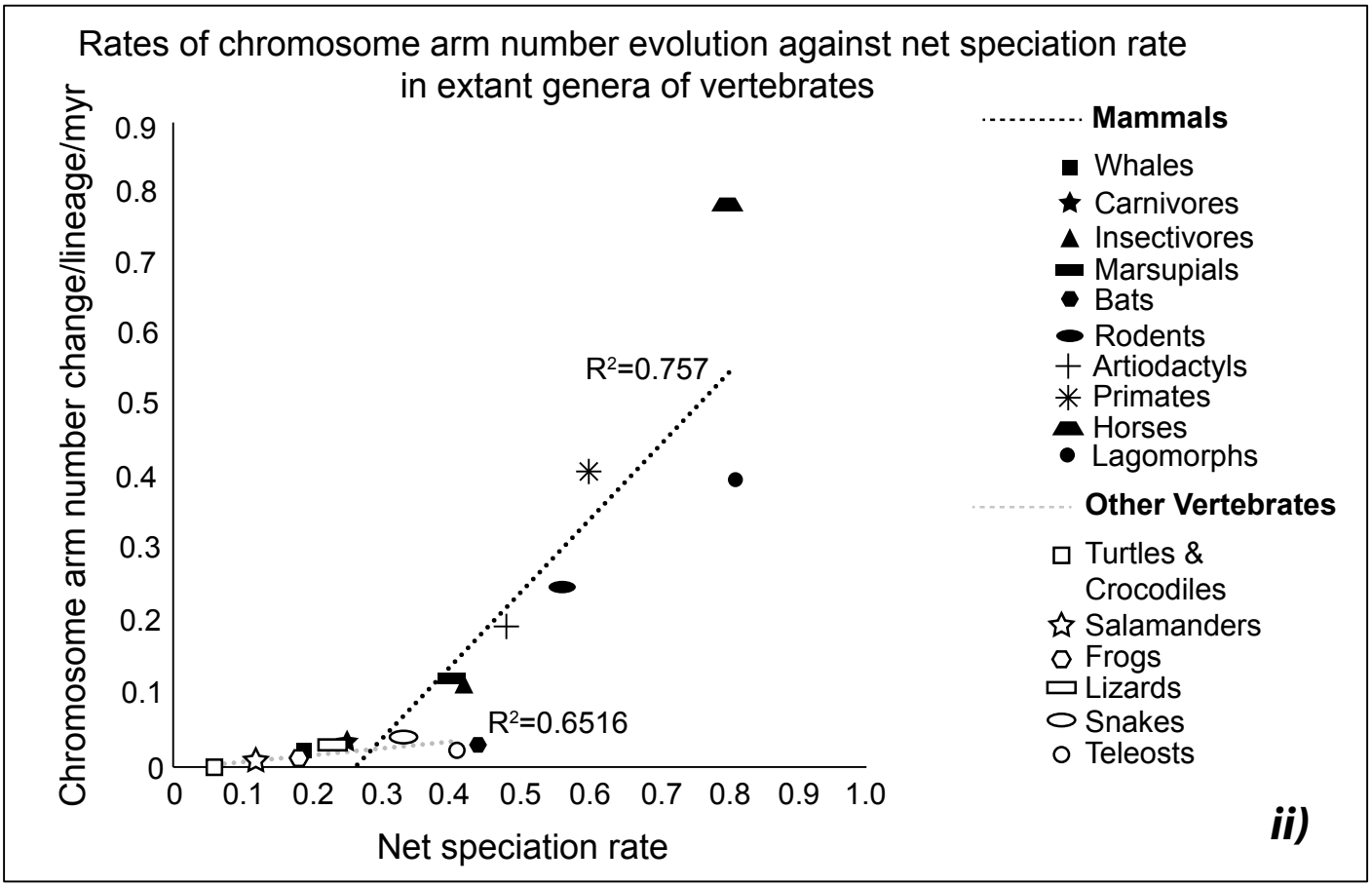


Figure 1. Key results from Bush *et al.* (1977). i) Rates of change in chromosome number of extant mammalian and other vertebrate taxa against net speciation rate. ii) Rates of chromosome arm number evolution of extant mammalian and other vertebrate taxa against net speciation rate. The R^2 statistics for these correlations are inset in each graph along with the trend lines for mammals (black dotted line) versus other vertebrates (grey dotted line).

strong genetic drift and increasing observations of within-species polymorphism for the same forms of chromosome change as were supposed to cause speciation (see also Sites and Moritz 1987; Coyne and Orr 2004). One exception is where multiple independent fusions establish in separate populations (via neutral processes) yet cause severe meiotic problems in hybrids if particular arms are involved in different fusions. This is called "monobrachial homology" and is a form of Dobzhansky-Muller incompatibility (Baker and Bickham 1986). Instead, Patton (2004) highlighted new theory and evidence (Navarro and Barton 2003) pointing to effects of chromosome change on the genomic distribution of recombination, with subsequent effects on accumulation of adaptive mutations within species and incompatibilities between species (see also Kirkpatrick and Barton 2006).

As Patton (2004) noted, the emerging ability to compare high-quality, de-novo whole genome assemblies reveals much more extensive chromosome change than was evident from numbers of chromosomes or chromosome arms that formed the basis of the analyses in Bush *et al.* (1977). Following his example, humans (2n = 46) and the other great apes (2n = 48) differ by just one fusion, yet recent genome comparisons reveal >17,000 fixed differences for structural variants (insertions, deletions and inversions) spanning (>18Mb) in humans on the branch from our common ancestor with chimpanzees, of which many are associated with changes in gene expression (Kronenberg *et al.* 2018). High-resolution analyses of copy number variants are providing important insights into genome change and speciation. For example, expansions of X-linked gene families associated with spermatogenesis ("ampliconic gene families") have an important role in reproductive isolation in apes and rodents (Kopiana *et al.* 2022).

Assessment of key insights from Bush *et al.* (1977)

Do mammals have a higher rate of chromosome change and is that correlated with a higher rate of speciation and with small population size? Recent compilations of cytogenetically characterised chromosome variation in mammals support the main findings of Bush *et al.* (1977). Using much more extensive data and phylogenetic – macroevolutionary comparisons, Martinez *et al.* (2017) confirmed wide (two orders of magnitude) variation in rates of macrochromosomal change among families of mammals and associations of high rates of chromosome change with geographic range and litter size. Using data from Martinez *et al.* (2017) and other sources, Herrick and Sclavi (2019) confirm a strong association between rates of chromosome change and speciation across orders and families of mammals. Interestingly, there was no correlation between degree of conservation of syntenic gene blocks and rates of chromosome change or speciation. Within diprotodontian marsupials, Westerman *et al.* (2010) contrasted strong conservation of the ancestral karyotype in several families with extensive chromosome change including fusion, fissions, centric shifts, in others, *e. g.* Macropodidae. A particular form of intrachromosomal rearrangement, centromere repositioning, occurs when neocentromeres establish to replace ancestral ones and is common in several cytogenetically variable mammalian taxa (reviewed in Brannan *et al.* 2024). There has been less attention to indicators of small population size, though for Carnivora, Jonicka *et al.* (2024) found that species with smaller ranges have a higher rate of Robertsonian translocations and small population size has been associated with higher rates of chromosomal rearrangement fixation in some groups (*e. g.* Australian rock-wallabies –*Petrogale*– see below). However, our ability to test this prediction and tease apart the role of genetic drift from selection remains a challenge.

The ongoing revolution in long-read sequencing has greatly improved the capacity to produce highly contiguous "chromosome scale" assemblies of genomes and so detect chromosome change in exquisite detail (Figure 2). The addition of HiC chromatin contact maps enables assembly of long-read scaffolds (because linked segments have more interactions) and, along with other methods (summarised in Mohan *et al.* 2024), reveals structure of chromatin in the interphase nucleus, including active (A, "euchromatin") or inactive (B, "heterochromatin") compartments and topologically associated domains (TADs, regions of coordinated gene expression). When combined with efficient algorithms to estimate ancestral genome arrangements (*e. g.* Muffato *et al.* 2023; Yu *et al.* 2024), comparing whole-genome assemblies provides rich, highly-resolved information on conserved syntenic regions, Evolutionary Breakpoint Regions (EBRs) at the boundaries of these regions and genome reshuffling at macroevolutionary scale. What does this rapidly accelerating knowledge tell us about the nature, cause and effects of chromosome rearrangements in mammals?

Comparisons of genome assemblies across vertebrates confirm the extraordinarily high rate of chromosome rearrangement in therian mammals relative to other amniotes – amphibians, birds, and non-flying reptiles (Waters *et al.* 2021; Bredeson *et al.* 2024). In a recent review, Damas *et al.* (2021) conclude that mammals are more prone to interchromosomal rearrangements and birds to intrachromosomal change. In a separate comparison of 92 vertebrate genome assemblies, including ancestral reconstructions, Muffato *et al.* (2023) again found that mammals have an elevated rate of interchromosomal rearrangements, with especially high rates in gibbons, dogs and murid rodents. They also inferred that rates of intrachromosomal rearrangements were similar across mammals and saurian reptiles, but higher in teleosts perhaps due to diploidisation in the latter.

Focussing on artiodactyls (*e. g.*, ungulates), another group for which Bush *et al.* (1977) reported a high rate of chromosome change, comparisons of whole genome assemblies found inversions to be the most common form of chromosome rearrangement, with fusions/fissions

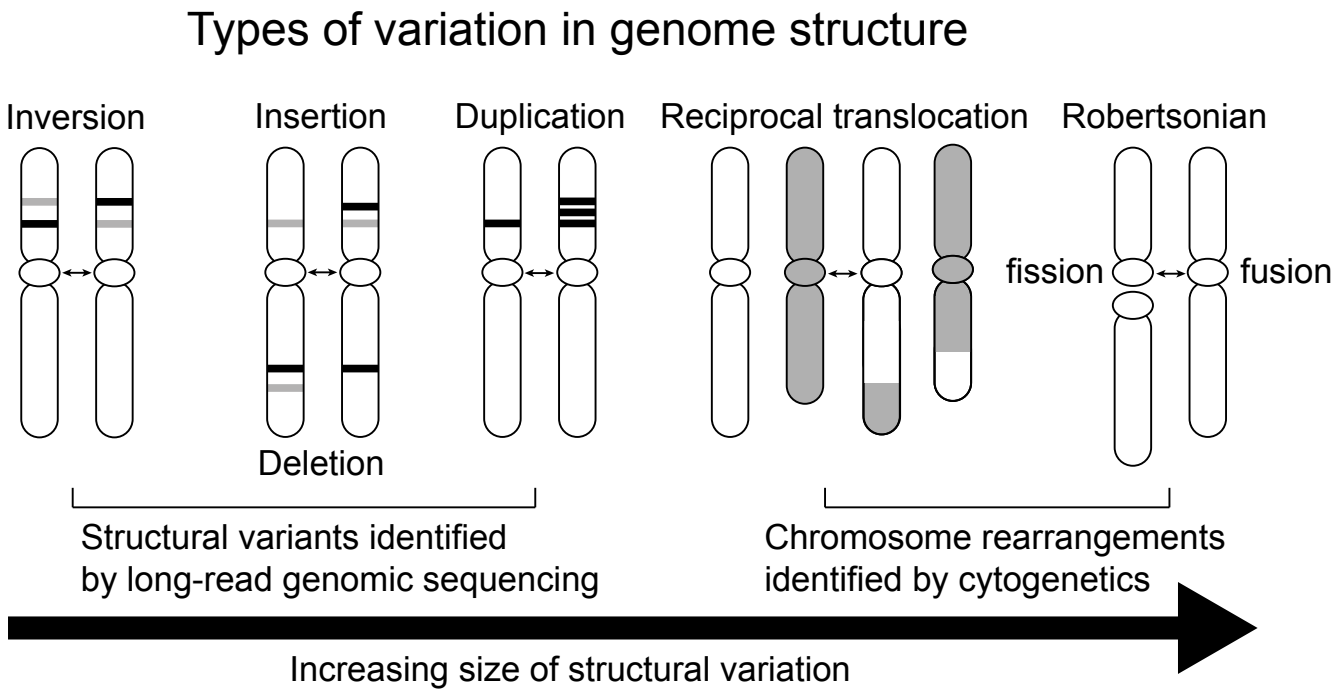


Figure 2. Expansion of types of genome rearrangements readily detected with direct assembly from long-read sequencing versus classical cytogenetics (excluding polytene chromosomes).

mostly occurring in ancestral lineages (Aria-Sarda *et al*. 2023). Muntjaks are a special case, with chromosome numbers varying from 2n = 46 to 2n = 6/7. Based on high-quality genome assemblies, Yin *et al.* (2021) estimated 31 fusion events (28 tandem and 3 Robertsonian) over the past ~ 2 My, but did not detect any increase in SNP divergence rates compared to Bovids with fewer chromosome rearrangements. Interestingly, the inferred pulse of fusions at ~ 1Myr corresponded with a period of reduced population size in the species with low chromosome numbers.

Do chromosome changes cause speciation? Bush *et al.* (1977; also White 1978; King 1993) summarised multiple ways by which chromosome rearrangements could initiate speciation. Recent reviews (Berdan *et al.* 2023; Lucek *et al.* 2023) support multiple effects –a given chromosome rearrangement can contribute to reproductive isolation and speciation via multiple, non-exclusive mechanisms– reduction in hybrid fitness (underdominance), change in recombination rate, and changes in gene expression. Meiotic drive can also contribute to establishment of chromosome rearrangements within populations. In parallel, there has been long-standing debate about whether chromosomal rearrangements that cause speciation always arise in isolated or allopatric populations, or can established in the face of gene flow and within the range of the ancestral form (reviewed in White 1978, King 1993). Most theory and evidence support allopatric origins of derived chromosome forms, though it is plausible that, via suppression of recombination, inversions could enhance adaptive speciation in parapatry. In contrast, there is little support for White's "Stasipatric" speciation model in which underdominant chromosome changes establish in sympatry because of increased fitness of homozygotes.

Underdominance. There is still limited evidence supporting strong underdominance due to single fusions or inversions as such mutations are unlikely to establish and/or are eliminated during gametogenesis (cells with univalents failing pachytene-diplotene transition or, for unbalanced products of recombination, shunted to polar bodies in oogenesis; Berdan *et al.* 2023). One exception is X-autosome translocations which cause substantial male sterility (Ashley 2002). However, heterozygotes for multiple chromosome rearrangements, as might accumulate via neutral processes in allopatry, can have substantially reduced fitness (Baker and Bickham 1986). Rock-wallabies are one example, in which such "monobrachial homology" occurs between some parapatric taxa and is expected to substantially reduce fitness of hybrids. The east coast *penicillata* group consists of eight parapatric and allopatric species which all have varied chromosome numbers due to Robertsonian fusions. Experimental crosses between species highlighted reduced fertility, which led to the description of three new species (Eldridge and Close 1992; Close *et al.* 1996). Haldane's Rule (Haldane 1922), where males are sterile and females are sub-fertile, is associated with divergence of species in this group and highlight potential mechanisms of a role of the sex chromosomes or meiotic drive in reproductive isolation. These organisms are restricted to complex rocky outcrops and have small population sizes, so it is thought chromosomal rearrangements could be fixed due to genetic drift alone. Theory would predict that if underdominance is the driver of divergence in such a system,

rearranged and non-rearranged regions of the genome would have comparable levels of genetic divergence. In contrast, if recombination suppression was the driving force for reproductive isolation, then we would expect the rearranged regions to have greater divergence than non-rearranged regions. Comparison of exonic sequences revealed higher sequence divergence on the X chromosome compared to autosomes, and differences between rearranged and non-rearranged regions of the genome, but not in the expected direction (Potter *et al.* 2022). Stronger tests of this hypothesis require full genome comparisons, using long-read sequencing, which is underway.

Chromosome rearrangements and recombination. There is now abundant evidence for suppression of recombination in polymorphic inversions within species and increased divergence between species or ecotypes in rearranged versus co-linear regions (Wellenreuther and Bernatchez 2018). For example, genes linked within a 41 Mb polymorphic inversion are responsible for multi-trait divergence between forest and prairie ecotypes of deer mice (Hager *et al.* 2022). In human-chimpanzee comparisons, recombination is reduced in segments spanning eight major inversion differences (Farre *et al.* 2012). Sequence divergence in inversions, which often span multiple genes, can be attributed to several, non-exclusive processes. These include adaptive divergence and accumulation of deleterious alleles, both of which are expected to occur over the life history of an inversion (Faria *et al.* 2018; Berdan *et al.* 2023), and each of which can lead to Dobzhansky-Mueller incompatibilities (Kirkpatrick and Barton 2006).

The impact of fusions on the recombination landscape has received less attention – but evidence is emerging of substantial effects. Theory predicts that recombination suppression could also lock up potentially adaptive loci around the fusion site (Guerrero and Kirkpatrick 2014). Marin-Garcia *et al.* (2024) studied wild mice populations with multiple fusions combining cytological analyses of meiotic configurations with linkage disequilibrium (LD) analyses of SNPs. Recombination in metacentrics shifted towards telomeres relative to acrocentrics leading to reduced recombination rate genome-wide and increased genomic divergence. Major shifts in recombination rates between all acrocentric and multiple-fusion populations was concentrated in regions with immune system and olfaction genes. However, recombination rates shifted primarily in response to genotype at a key gene (Prdm9), with effects of fusions per se restricted to centromeric regions.

Adaptive advantage of chromosome rearrangements. White (1978) and other proponents of underdominance as a primary driver of chromosomal speciation suggested that newly arisen chromosome changes might have adaptive advantages that would mitigate reduced fertility in heterozygotes. This could happen via position effects, where gene expression is altered at or near the breakpoints, or positive epistasis between newly linked genes. One such example is the effect of a Robertsonian translocation on expression of genes associated with muscle development in the gayal (*Bos frontalis*) which lives in rugged mountains (Li *et al.* 2023). Otherwise, most evidence for positively selected rearrangements comes from analysis of large inversions that are segregating within species and are associated with ecological divergence (Berdan *et al.* 2023). High-resolution analyses of rearrangements in several species indicates that few breakpoints occur at boundaries of Topologically Associating Domains (TADs; see below), suggesting that most surviving changes have limited effect on gene expression. Across great apes and humans, deletions at TAD breakpoints are selected against, especially in association with strong transcription start sites (Fudenberg and Pollard 2019). Clearly, there is much more to be learnt here, especially as studies combining high resolution mapping of chromosome rearrangements and effects on gene expression are expanded to more systems.

Meiotic drive. As only one of four products of meiosis survive as a mature oocyte (the rest being relegated to polar bodies), variation in the strength of centromere interactions with the asymmetric spindle of the oocyte can cause specific forms of chromosomes to increase in frequency in a population. This is called meiotic drive and has long been recognised as a mechanism that could enable establishment of underdominant rearrangements (White 1978; Hedrick 1981; Walsh 1982). Across mammals, where Robertsonian fusions or centric shifts are common, karyotypes of most species are mostly acrocentric or metacentric, with a marked deficit of species with intermediate states (Pardo-Manuel de Villena and Sapienza 2001). This pattern is attributed to meiotic drive in which either metacentric or acrocentric morphologies are favoured, combined with frequent shifts in the direction of drive. When the direction of drive does change, it is expected that the rate of change in chromosome number will increase towards whatever state is now favoured. This prediction was supported using novel macroevolutionary models (Blackmon *et al.* 2019). The meiotic drive hypothesis is supported by extensive experimental evidence in mice, humans and birds (Pardo-Manuel de Villena and Sapienza 2001) and is now well understood mechanistically (*e. g.* for mice, Akera *et al.* 2017).

Why do taxa differ in the rate and form of chromosome rearrangements? It has long been recognised that the form, as well as rate, of chromosome change differs at phylogenetic scale. This is evident in the data from Bush *et al.* (1977) and was termed karyotypic orthoselection by White (1973; see also King 1993). The hope is that our rapidly increasing knowledge of genomic characteristics of evolutionary breakpoint regions (EBRs) and of chromatin architecture during interphase can shed some light. EBRs are typically depleted in coding genes, occur between rather than within TADs, and in some, but not all (Arias-Sardia *et al.* 2023) cases, are associated with expansions of repeated sequences including Transposable Elements (TEs; Yin *et al.* 2021; reviewed in Brannan *et al.* 2024). That mobilisation of TEs can lead to bursts of chromosome rearrangement is a

long-standing hypothesis (Lawson *et al.* 2023) and there are now some clear examples relating expansions of particular TEs to elevated rates of chromosome reorganisation in mammals (O'Neill *et al.* 1998 in Macropods; Carbonne *et al.* 2014 for Gibbons; Sotero-Caio *et al.* 2015 in Phyllostomatid bats), presumably via non-homologous recombination. In the case of the Gibbons, insertions of the novel retrovirus were also shown to modify expression of genes controlling chromosome segregation.

These above examples aside, consistent differences in characteristics of EBRs between taxa with high rates of interchromosomal vs intrachromosomal rearrangements have proved elusive (Brannan *et al.* 2024). An alternative hypothesis – the Integrative Breakage Model (Farre *et al.* 2015) posits that propensity towards different forms of rearrangement relates to how chromatin is organised during interphase of germ-line cells. Álvarez-Gonzalez *et al.* (2022) explored this hypothesis using new evidence on 3D genome organisation (*e. g.* via HiC) across therian mammals. Eutherians have chromosomes organised in spatially distinct territories regardless of chromosome number whereas marsupials have clustered ("Rabl" form) centromeres. The resulting increased opportunity for inter-chromosomal interactions of chromatin territories in eutherians could explain higher rate of interchromosomal rearrangements. Marsupials have fewer, larger chromosomes with more intra-chrom rearrangements. No doubt, there is much more to learn here via integrated analyses of genome structure, chromatin organisation and gene expression across related species (Figure 3; Mohan *et al.* 2024).

Moving forward. Advances in genomic sequencing technologies now enable us to identify finer-scale structural variation (*e. g.*, Figure 2) and we can now see that structural variation can occur at different scales, with different consequences of pre- or post-zygotic incompatibilities resulting in speciation (Berdan *et al.* 2023). But it is only with a multi-omics approach (*e. g.*, chromatin structure, transcriptomics and epigenomics) that we will be able to piece together the consequences and mechanisms of structural variation evolution and outcomes on fitness/divergence (Figure 3). As well as taking this broad omics approach, we need to be mindful about the proposed connections to population-level processes of recombination, selection and drift. As recent works on model systems demonstrate, there is also exciting potential to scale from experimental manipulations at cellular scale to organismal and population processes (Ansai and Kitano 2022). As Bush *et al.* (1977) recognised, the greater the comparative breadth of insight we have across mammals, the more we can learn about what drives chromosome change and how this promotes organismal diversity.

Fortunately, with new theory and tools, the questions about why vertebrate taxa differ in rates and forms of

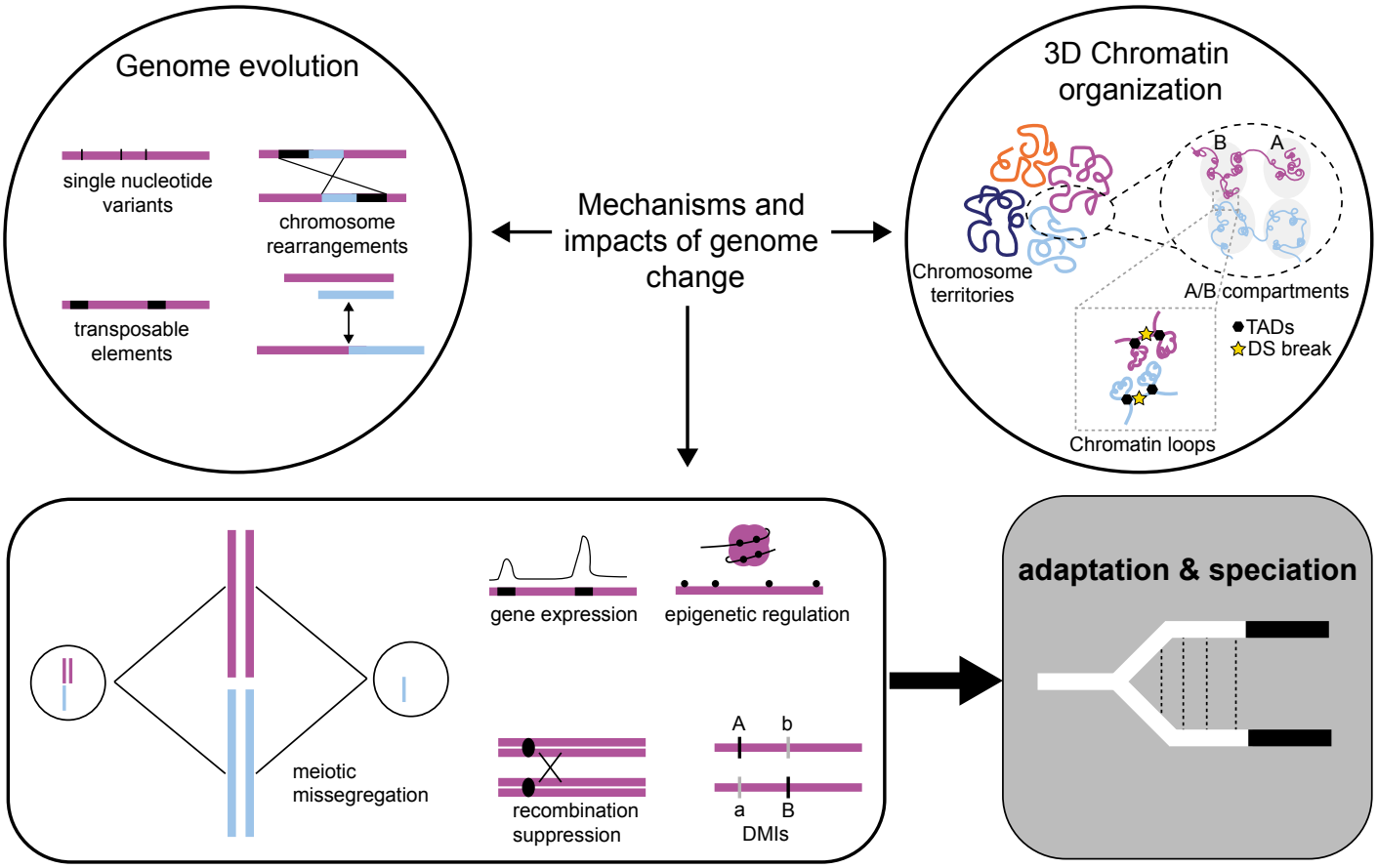


Figure 3. Integration of analyses of genome structure, chromatin organisation, gene function and recombination landscapes across closely related species is a promising new direction. Figure modified from Mohan *et al.* (2024)

chromosome change, and how all this relates to speciation remain hot topics. Classical cytogenetics left us with a rich diversity of systems to explore and it is now possible to compare genomes, chromatin organisation, gene expression and historical demography across such taxa. Bush *et al.* (1977) was a great start and there is much left for Jim Patton to ponder!

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Species boundaries, hybridization and gene flow

Michael W. Nachman[1]

[1]Museum of Vertebrate Zoology and Department of Integrative Biology. University of California, Berkeley, Berkeley. California 94720, United States of America. Email: mnachman@berkeley.edu.
* Corresponding author: https://orcid.org/0000-0003-4321-5135.

The rise of genomics has spurred a renewed interest in hybridization and the permeability of species boundaries. However, these ideas are not new. Here I review early work by Patton and colleagues on hybridization, gene flow, and the nature of species boundaries in pocket gophers and argue that a focus on the underlying biology of the organism provides insights into hybridization and gene flow that are not obtainable from genomic data alone.

El auge de la genómica ha estimulado un renovado interés en la hibridación y la permeabilidad de los límites entre especies. Sin embargo, estas ideas no son nuevas. En este artículo, analizo los primeros trabajos de Patton y sus colegas sobre la hibridación, el flujo genético y la naturaleza de los límites entre especies en las tuzas y sostengo que un enfoque en la biología subyacente del organismo proporciona conocimientos sobre la hibridación y el flujo genético que no se pueden obtener a partir de datos genómicos únicamente.

Keywords: Population genetics; introgression; pocket gophers; *Thomomys bottae*.

The publication of a Neandertal genome sequence in 2010 included the startling discovery that most modern humans carry a small percentage of Neandertal DNA as a result of hybridization when the two species came into contact (Green *et al.* 2010). This conclusion was reached using a then-new statistical test (the ABBA-BABA test) that could be applied to whole-genome data to distinguish between shared variation due to unsorted ancestral polymorphism and shared variation due to gene flow. This discovery was followed by a torrent of papers showing that hybridization is common in many taxa and that species boundaries are permeable (Payseur and Rieseberg 2016). Hybridization became fashionable.

Of course, these topics are not new, and the recent studies were preceded by decades of empirical research on hybridization in a variety of taxa (*e. g*. Darwin 1859; Endler 1977; Arnold 1992). Jim Patton has been studying species boundaries, hybridization, and gene flow in gophers and other small mammals since the 1960's (Patton and Dingman 1968). Here I describe some of that work and highlight a few papers which I consider to be superb and which had a strong influence on my own research and thinking about hybridization and gene flow.

In a wonderful series of papers on pocket gophers over several decades, Patton documented morphological, cytogenetic, and genetic variation across Western North America in the genus *Thomomys* (*e. g*. Patton and Dingman 1968; Patton *et al.* 1972; Patton 1973; Patton and Yang 1977; Patton *et al.* 1979; Patton and Sherwood 1982; Hafner *et al.* 1983; Patton *et al.* 1984; Smith and Patton 1984; Patton and Smith 1990; Patton and Smith 1994). Most of this work focused on *T. bottae* but also included the closely related species *T. townsendii* and *T. umbrinus* with which *T. bottae* hybridizes. Patton was an early adopter of using starch gel electrophoresis to measure genetic variation as reflected in differences in allozyme frequencies (Patton *et al.* 1972), and he discovered that conspecific populations of *T. bottae* exhibited unusually high levels of genetic differentiation (Patton and Yang 1977). In fact, *T. bottae* is unusual among mammals in harboring an enormous amount of variation in karyotype, genes, and morphology. For example, within *T. bottae*, there are over 100 recognized morphological races (Patton and Smith 1990). This extreme variation stems from a variety of factors, including the large geographic range of *T. bottae* and the wide diversity of habitats and environments in which the species is found, from sea level to 13,000 feet. In addition, a fragmented distribution and comparatively low levels of dispersal provide opportunities for differentiation among populations. Patton was interested in describing the extent of hybridization among these different forms, as well as the amount of hybridization between *T. bottae* and closely related species where they come into contact.

Much of the initial interest in studying patterns of hybridization in gophers came from a desire to delineate species boundaries, rather than to study hybrid zone dynamics per se (Patton 1993). Nonetheless, these studies provided important insights into the frequency and consequences of hybridization among genetically differentiated taxa. Viewed through the lens of recent genomic studies of hybridization, Patton's work stands out for its deep focus on the biology of the organism. Pocket gophers are subterranean mammals, and much of their biology follows from this lifestyle. They are restricted to friable soils and consequently often have patchy distributions. They are solitary and live at low densities. Where taxa come into contact, they have mainly parapatric rather than overlapping distributions.

One of the clear patterns to emerge from Patton's work is that despite high levels of morphological and genetic differentiation between parapatric forms, hybridization is common where taxa meet. Most hybrid zones are narrow relative to the range of the hybridizing taxa, reflecting relatively low densities, limited movements, and restricted habitats available to gophers. Despite the prevalence of narrow hybrid zones, the consequences of hybridization differ substantially among different parapatric forms. For example, in central New Mexico *T. bottae ruidosae* and *T. b. actuosus* meet and hybridize along Nogal Canyon. These forms differ substantially in karyotype, morphology, and allozymes, with three fixed allozyme differences. Few F1 individuals are observed, but multiple backcross or later-generation intercross animals are seen (Patton *et al.* 1979). Moreover, evidence of mismatched allozyme alleles (in both directions) are seen in populations many miles from the narrow contact zone, consistent with significant introgression. In contrast, *T. bottae* and *T. umbrinus* hybridize in Sycamore Canyon in the Patagonia Mountains of southern Arizona. Again, the hybridizing forms differ substantially in karyotype, morphology, and allozymes, with three fixed allozyme differences. However, in this case, nearly all hybrids are F1's with little evidence of backcross progeny (Patton 1973). Moreover, histological studies of testes in F1 males reveal that these animals are either sterile or have greatly reduced fertility. Comparison of these two different hybrid zones (as well as others) illustrated the important and surprising result that the amount of genetic differentiation is not a good predictor of the amount of isolation in gophers. While the recent genomic data reveal a history of introgression between many taxa, such data tell us little about what actually happens when two taxa meet.

Patton recognized that species boundaries are difficult to define not only because of hybridization but also because the sorting of ancestral polymorphism and the genealogical relationships of small local populations can lead to biological species that are not monophyletic. In a series of papers, Patton and Smith (1981, 1989, 1994), along with work by Thaeler (1980) and Rogers (1991), provided an early empirical example that added to a growing recognition of the potential discordance between gene trees and species trees from both theory (*e. g.* Tajima 1983; Hudson 1992) and data (Avise *et al.* 1983; Avise 1989). This work anticipated the now-widespread understanding from genomic data that different genes may produce discordant trees (*e. g.* Degnan and Rosenberg 2009). *T. bottae* and *T. townsendii* have non-overlapping distributions in the northern Great Basin, and they hybridize in the Honey Lake Valley of northern California. Several studies showed that *T. bottae* is paraphyletic with respect to *T. townsendii*; in other words, there are some populations of *T. bottae* that are more closely related to *T. townsendii* than they are to other populations of *T. bottae* (Thaeler 1980; Patton and Smith 1989; Rogers 1991). Notably, Patton and Smith (1994) discovered not only that *T. bottae* is paraphyletic, but that different genes (mitochondrial and nuclear) revealed different phylogenies among the populations of these species, providing conflicting views of monophyly, paraphyly, and even polyphyly for species. These conclusions were possible only because of the detailed sampling that was performed as well as the use of different molecular markers, something that was still relatively uncommon in the early 1990's. Two quotes from this paper of 30 years ago seem particularly prescient.

"The complexities uncovered in this particular example are probably similar in virtually all other groups of pocket gophers and many other organisms. In other words, this case history is not likely to be an isolated incident, which can thus be ignored; rather, this pattern may be commonly observed for a wide range of organisms." And later: "… the more one knows about variation within and among populations, and thus the more detail that is available regarding intraspecific genealogy, the more likely it will be that the boundaries of species will be blurred…" (p. 23, Patton and Smith 1994). Indeed, blurry species boundaries now seem commonplace.

One of my favorite papers by Patton involves the study of gene flow among local populations of pocket gophers that are geographically close and not reproductively isolated. Conducted with his postdoc, Joanne Daly, this paper stands as one of the more thorough studies of gene flow in any mammal estimated using both direct and indirect methods (Daly and Patton 1990). In this study, they followed the movement of individual gophers between fields at the Hastings Natural History Reservation in Carmel Valley, California over several years using an impressive combination of approaches. All gophers were tagged, and blood was taken from all animals. Every animal in each of several populations was tracked. Gophers were trapped above ground using pitfall traps, and underground in burrows. Dispersal was recorded between established populations, as well as into fields in which all gophers had been removed. Observations were made almost continuously for eight months out of the year, for three consecutive years. The sex, age, and reproductive condition of all animals were recorded. Finally, all of the direct measures of dispersal were compared to indirect inferences of gene flow obtained from analysis of patterns of genetic differentiation from allozyme data generated in this study combined with data from a previous study of the same populations (Patton and Feder 1981). This combination of approaches revealed many insights into the nature of gene flow between local populations. For example, gophers often disperse above ground. Females disperse when young, but males disperse later. Notably, genetic data suggested that 8 to 18 migrants moved between populations each generation, a number substantially *above* the 1 to 6 individuals that were observed to be dispersing between established populations, but substantially *below* the 20 to 40 individuals that moved into unoccupied habitat. These observations are consistent with the idea that gene flow may occur from a combination of recolonization following local extinction as

well as dispersal between established populations. Such a conclusion would have been impossible without the combination of direct and indirect approaches used by Daly and Patton. Another insight to emerge from this work is that even though females may disperse more than males, males may contribute more to gene flow through a heavily biased operational sex ratio. These and many other insights about dispersal and gene flow were possible because of an intense focus on individual animals in the field, insights that are not obtainable from genomic data alone.

Jim Patton is known to many as a preeminent mammalogist. From my brief comments on just a few of his many excellent papers, I hope to have conveyed that he also made lasting contributions to our understanding of basic issues in population genetics and evolutionary biology.

Jim Patton has had a profound influence on me both professionally and personally. I was fortunate to be an undergraduate at UC Berkeley in the early 1980s and to take many of the "ology" classes that were offered. Berkeley continues to offer these important classes, placing a premium on the experience that students get when exposed to field work and studies of organisms in their natural environment. The most memorable class of my college career was Jim Patton's mammalogy class. I was captivated by the science and by Jim's enthusiasm as a teacher. His class was rigorous and demanding, and as a professor, he was approachable. He always had high expectations, and students seemed to rise to the challenge. Like many people who teach mammalogy, I have modeled my own class after his. I was thrilled when he and Doug Kelt revised Lawlor's "Handbook to the Orders and Families of Living Mammals." We use the collections of the MVZ in my class, and my students are always amazed, as am I, by the sheer number of specimens with JLP tags on them, representing Orders and Families of mammals from every continent.

After taking his mammalogy class as a young student, I approached Jim to ask if I could get involved in research. He asked what I wanted to study. I said that I had no idea. He said something like "well, go figure it out and then come back!" Undeterred, I started talking with his graduate students and eventually ended up doing a little project on kangaroo rats. A few years later I had the unforgettable experience of joining him and Carol in the field in the altiplano of southeastern Perú. Jim has achieved a near-mythical status as a field biologist, and all I can say is that all the stories are true.

The aspect of Jim that I most admire is his support for others and his unfailing generosity. He is never too busy to stop and help others, and he treats everyone with the same kindness and respect, from young undergraduates to senior colleagues. He is, quite simply, a gem of a human. My experience is not unique – I know that Jim influenced generations of students and colleagues, and that our lives are richer because of him.

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Cytogenetic diversity of non-volant small mammals in the Serra dos Órgãos region, Rio de Janeiro state, Brazil

Lena Geise[1]*, Luciana Guedes Pereira[2], Marianne Bello[3], and Marcia Aguieiras[1]

[1] Departamento de Zoologia, Universidade do Estado do Rio de Janeiro. Rua São Francisco Xavier, 524. Rio de Janeiro, RJ, Brazil. Email: lenageise@gmail.com; lena.geise@uerj.br (LG) and m.r.aguieiras@gmail.com (MA).

[2] KEO International Consultant. Avenida, Dom João II, 11, décimo andar, 1990-221. Lisboa, Portugal. Email: luciana@gpereira.bio.br (LGP).

[3] Programa de Pós-Graduação em Ecologia e Evolução, Departamento de Ecologia, Universidade do Estado do Rio de Janeiro. Rua São Francisco Xavier, 524. Rio de Janeiro, RJ, Brazil. E-mail: mariannesbello@gmail.com; bello.marianne@posgraduacao.uerj.br (MB).

* Corresponding author: https://orcid.org/0000-0003-1239-8047.

The Atlantic Forest is one of South America's most biodiverse regions, hosting a significant portion of Brazil's small non-volant mammal diversity, including 267 rodent and 66 marsupial species. The Serra dos Órgãos region in Rio de Janeiro state is a key area for studying this diversity, as it houses 32 rodent and 13 marsupial species. Rodents, unlike marsupials, exhibit a high diversity of chromosomal forms, which serve as important taxonomic tools for identifying cryptic species. Our study used cytogenetic analyses to enhance the taxonomic resolution and understanding of small mammal biodiversity in the Serra dos Órgãos, focusing on the high chromosomal variation in rodents, particularly those within the Sigmodontinae. We collected and karyotyped specimens from 25 localities within the municipalities of Cachoeiras de Macacu, Guapimirim, Petrópolis, and Teresópolis in Rio de Janeiro. These areas include montane and lowland regions of the Serra dos Órgãos, ranging from 100 to 2,100 meters in altitude. Specimens were captured using live traps and handled following ethical guidelines, with karyotypic analysis performed on metaphase chromosomes obtained from bone marrow cell cultures. We analyzed 220 specimens, representing 20 rodent and five marsupial species. Significant intraspecific chromosomal variation was observed in seven rodent species, particularly within the sigmodontines. *Akodon cursor* displayed variation in fundamental numbers, while *Brucepattersonius nebulosus* exhibited variation in both diploid and fundamental numbers. New karyotypes were identified for the echimyid *Phyllomys* spp.. Our findings underscore the importance of cytogenetic analyses in revealing cryptic species and enhancing taxonomic resolution among South American rodents. The chromosomal variation observed highlights the need for integrating cytogenetic data to understand the evolutionary dynamics and biodiversity of the Atlantic Forest.

La Mata Atlántica es una de las regiones más biodiversas de América del Sur, albergando una parte significativa de la diversidad de mamíferos pequeños no voladores de Brasil, incluidos 267 especies de roedores y 66 de marsupiales. La región de Serra dos Órgãos en el estado de Río de Janeiro es un área clave para estudiar esta diversidad, ya que alberga 32 especies de roedores y 13 de marsupiales. Los roedores, a diferencia de los marsupiales, exhiben una alta diversidad de formas cromosómicas, que sirven como herramientas taxonómicas importantes para identificar especies crípticas. Nuestro estudio tiene como objetivo utilizar análisis citogenéticos para mejorar la resolución taxonómica y la comprensión de la biodiversidad de los pequeños mamíferos en la Serra dos Órgãos, centrándose en la alta variación cromosómica en los roedores, particularmente en aquellos dentro de la subfamilia Sigmodontinae. Recogimos y cariotipamos especímenes de 25 localidades dentro de los municipios de Cachoeiras de Macacu, Guapimirim, Petrópolis y Teresópolis en Río de Janeiro. Estas áreas incluyen regiones montañosas y de tierras bajas de la Serra dos Órgãos, que van desde los 100 hasta los 2,100 metros de altitud. Los especímenes fueron capturados utilizando trampas vivas y manejados siguiendo pautas éticas, con análisis cariotípicos realizados en cromosomas metafásicos obtenidos de cultivos de células de médula ósea. Analizamos 220 especímenes, representando 20 especies de roedores y cinco de marsupiales. Se observó una variación cromosómica intraespecífica significativa en siete especies de roedores, particularmente dentro de la familia Cricetidae. *Akodon cursor* mostró variación en los números fundamentales, mientras que *Brucepattersonius nebulosus* exhibió variación tanto en los números diploides como en los fundamentales. Se identificaron nuevos cariotipos para *Phyllomys* spp.. Nuestros hallazgos subrayan la importancia de los análisis citogenéticos para revelar especies crípticas y mejorar la resolución taxonómica entre los roedores sudamericanos. La variación cromosómica observada resalta la necesidad de integrar datos citogenéticos para comprender las dinámicas evolutivas y la biodiversidad de la Mata Atlántica.

Keywords: Atlantic Forest; Didelphimorphia; intraspecific variation; karyotypes; Rodentia.

Introduction

Among the small non-volant mammals of Brazil, rodents exhibit the greatest diversity, with 267 described species; marsupials are less diverse, with 66 recognized species (Abreu *et al.* 2023). The Atlantic Forest, considered one of the most diverse regions in South America, harbors a significant portion of this Brazilian diversity (Prado *et al.* 2015), including 110 rodent and 24 marsupial species. This includes species from open areas at the border of the Atlantic Forest (Brandão and Hingst-Zaher 2021). The Serra dos Órgãos region represents one of the few remaining areas of biodiversity in the state of Rio de Janeiro (Cronemberger

and Castro 2009). Serra dos Órgãos National Park houses 32 species of rodents and 13 species of didelphimorph marsupials (Cronemberger *et al.* 2019).

Unlike didelphimorphs, rodents exhibit a high diversity of chromosomal forms, even within the same genus (*e. g.*, *Akodon*: Brandão *et al.* 2021). This variation is evident in diploid and fundamental numbers as well as in the distribution of heterochromatin blocks and other important cytogenetic features (Romanenko and Volobouev 2012). The significant number of karyotype descriptions highlights this high cytogenetic variation, which serves as an important taxonomic tool, especially for genera that contain cryptic species (*e. g.,* Pardiñas *et al.* 2015; Tribe 2015). Therefore, karyotypic information contributes importantly to the characterization of biodiversity, both at continental scales and within more restricted regions, whether at the individual, population, or higher taxonomic levels (Patterson and Costa 2012). According to Paresque *et al.* (2018), most karyotypes of Brazilian rodents and marsupials have been available since frequent publication of these data began in the 1970s. However, additional data, particularly when from a considerable number of individuals of the same species from the same locality, offer valuable insights into intraspecific and geographic variation.

The collection efforts of small mammals in the Serra dos Órgãos range began in the 1990s. In 1991, two taxa – *Delomys dorsalis* and, at the time, a *Rhipidomys* sp. – were collected in Garrafão (locality 16 of the present work; Figure 1) and karyotyped (L. Geise, personal communication). A long-term study began in 1996, primarily coordinated by the Laboratório de Vertebrados (LabVert, Ecology Department, Universidade Federal do Rio de Janeiro; Gentile and Kajin 2015). This study allowed numerous genetic analyses (Aguieiras *et al.* 2013; Maestri *et al.* 2016; Pardiñas *et al.* 2016; Malcher *et al.* 2017; Paixão *et al.* 2021), the description of a new species (*Rhipidomys itoan*; de Andrade *et al.* 2011), and a comprehensive species list with their areas of occurrence (Cronemberger *et al.* 2019). Here, we present a review and broad description of several specimens collected over the past 25 years that have allowed for the acquisition of novel karyotypes.

Materials and methods

The specimens analyzed for karyotypic data were collected from 25 localities within the municipalities of Guapimirim, Cachoeiras de Macacu, Petrópolis, and Teresópolis, all situated in the state of Rio de Janeiro (Figure 1). These localities are located on the Atlantic slope of the Serra do Mar, encompassing both the hills and lowlands of the Serra dos Órgãos. The mountainous regions (Petrópolis and Teresópolis) are covered by dense submontane and montane rainforest (Rizzini 1954). In contrast, the lowland regions (Guapimirim and Cachoeiras de Macacu) contain fragments of dense ombrophilous forest (tropical rainforest) that are surrounded by pastures or plantations (Cabral and Fiszon 2004). Locations within the Serra dos Órgãos National Park (hereafter PARNASO) range from 400 to 2,100 meters, while those in the fragmented lowland areas are between 100 and 200 meters in altitude. Small terrestrial mammals were collected using live traps (Sherman, Tomahawk, or similar) placed on the ground, in the understory (one to two meters high), or in the canopy (on platforms at least six meters high) from 1991 to 2018.

The small mammals captured were brought to the laboratory and handled according to protocols approved by the American Society of Mammalogists (Sikes *et al.* 2016). Voucher specimens for all karyotypes were deposited in the mammal collections of Laboratório de Mastozoologia (LabMast, Zoology Department, State University of Rio de Janeiro) and the Museu Nacional (UFRJ). Preliminary identification was based on primary external morphological characteristics. Taxonomic nomenclature follows Astúa (2015) and Faria *et al.* (2019) for Didelphimorphia, and Patton *et al.* (2015), Abreu-Júnior and Percequillo (2019), and Abreu *et al.* (2023) for Rodentia. This taxonomic arrangement differs from https://www.mammaldiversity.org/taxa.html for *Guerlinguetus brasiliensis* but is in accordance with Abreu *et al.* (2023).

Metaphase chromosomes were obtained from bone marrow cell cultures following the protocol of Geise (2014), with some preparations including the addition of ethidium bromide. Metaphase preparations were spread on slides, stained with a 5 % Giemsa solution, and examined using a trinocular optical microscope, model Eclipse 50i. For each slide, at least 20 metaphase chromosomes were examined to obtain high-resolution images that allowed for the determination of diploid number (2n), fundamental number (FNa), and chromosomal morphology. Photographs were taken using a Nikon Digital Color DS-Fi1 camera attached to the microscope. Karyotypes were assembled starting with two-armed chromosomes in order of decreasing size, followed by acrocentric chromosomes; each assembly was compared with karyotypes described previously in the literature. The autosomal complement and sex chromosomes were distinguished using the chromosome nomenclature based on centromere position proposed by Levan *et al.* (1964). Sex chromosomes were identified according to the literature (Table 1). Almost all chromosomal preparations were deposited in the LabMast collection (Geise and Aguieiras 2021).

Results

A total of 220 specimens were karyotyped, comprising 20 rodent species and five marsupials. Among rodents, Cricetidae was the most diverse group sampled, being represented by 15 species from four tribes and one *incertae sedis* of Sigmodontinae. Other taxa sampled included Echimyidae (three species), Muridae and Sciuridae (one species each; Table 1 and Supplementary Material). *Akodon cursor* (64 specimens / 15 localities) and *A. montensis* (24 specimens / 5 localities) were the most intensely sampled

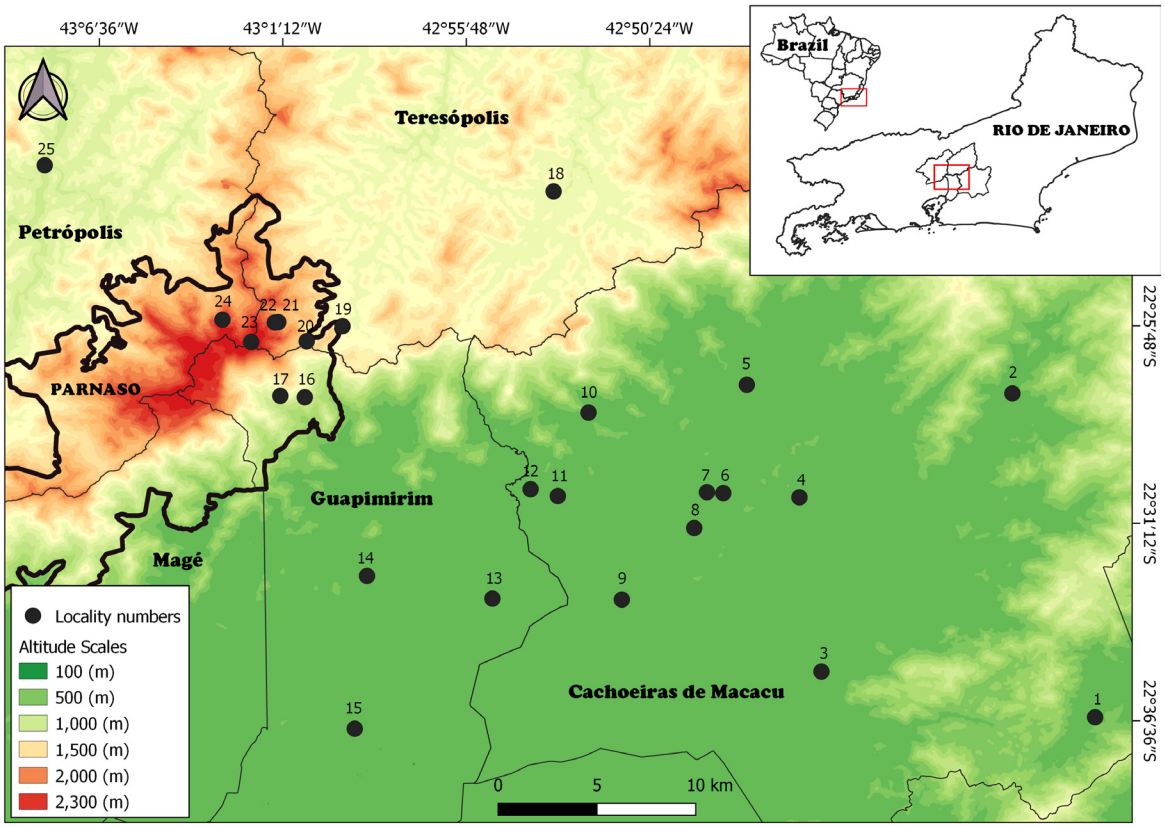

Figure 1. Collection localities for all karyotyped small terrestrial mammals in the Serra do Mar, Rio de Janeiro state. Lines indicate municipality limits. Colors represent the altitudinal scales, from green (lower altitudes) to red (higher altitudes). Red boxes in both Brazil and Rio de Janeiro State delineate the study area. Locality numbers refer to: Cachoeiras de Macacu Municipality: 1. Fazenda Nova Miracema (-22° 36' 33.7" S, -42° 36' 11.6" W, 102 m); 2. Fragmento (-22° 27' 50" S, -42° 39' 10" W, 68 m); 3. Fazenda Santo Estevão e Propriedade do Sr. Edimar (-22° 35' 44" S, -42° 44' 20" W, 100 m); 4. Fazenda Pica Pau Amarelo (-22° 31' 00" S, -42°45' 16" W, 200 m); 5. Reserva Ecológica de Guapiaçu (-22° 28' 00" S, -42° 46' 60" W, 34 m); 6. Conjunto de Fazendas (-22° 31' 00" S, -42° 47' 31" W, 100 m); 7. Fazenda Sem Nome (-22° 31' 00" S, -42°48' 00" W, 150m); 8. Fazenda Parahy (-22° 32' 00" S, -42°48' 19" W, 150 m); 9. São José da Boa Morte (-22° 34' 4" S, -42° 50' 20" W, 100 m); 10. Sítio Rosimery (-22° 29' 00" S, -42°51' 37" W, 200 m); 11. Estação Ecológica do Paraíso (-22°31' 19.80" S, -42°52' 23.20" W, 68 m). Guapimirim Municipality: 12. Fazenda Iguaçu (-22° 31' 11" S, -42°53' 12" W, 100 m); 13. Fazendas Consorciadas (-22° 34' 14" S, -42° 54' 9" W, 150 m); 14. Fazenda Chorona (-22° 33' 48" S, -42° 57' 53" W, 200 m); 15. Centro de Primatologia do Rio de Janeiro (INEA) (-22° 38' 00" S, -42° 58' 00" W, 100 m); 16. Parque Nacional da Serra dos Órgãos (PARNASO), Garrafão (-22° 29' 00" S, -43° 00' 00" W, 700 m); 17. PARNASO, Subsede, Município de Guapimirim (-22° 29' 00" S, -43° 00' 43.48" W, 400 m). Teresópolis Municipality: 18. Fazenda Boa Fé (-22° 23' 00" S, -42° 53' 00" W, 880 m); 19. PARNASO, Sede (-22° 27' 00" S, -42° 59' 00" W, 1,200 m); 20. PARNASO, Rancho Frio (-22° 27' 28" S, -43°00' 2" W, 1,200 m); 21. PARNASO, Trilha do Sino, Parte Baixa (-22° 26' 59" S, -43° 00' 54" W, 1,627m); 22. PARNASO, Abrigo 4 (-22° 27' 00" S, -43°01' 00" W, 2,130 m); 23. PARNASO, Pedra do Sino (-22° 27' 34" S, -43° 01' 40" W, 2,100 m). Petrópolis Municipality: 24. PARNASO, Vale das Antas, Município de (-22° 27' 00" S, -43° 02' 33" W, 1950 m); 25. Fazenda Inglesa (-22° 23' 00" S, -43° 08' 00" W, 910 m).

species; we karyotyped 73 (from 15 localities) and 34 (from four localities) specimens, respectively. Table 1 provides a detailed summary of all species karyotyped, number of individuals analyzed, observed variation in diploid and fundamental numbers, and form of the sex chromosome pair.

No intraspecific chromosomal variation was detected in the Didelphimorphia sample, which included *Caluromys philander* (2n = 14, FNa = 20), *Gracilinanus microtarsus* (2n = 14, FNa = 20), *Marmosops incanus* (2n = 14, FNa = 24), *Monodelphis scalops* (2n = 18, FNa = 30), and *Philander quica* (2n = 22, FNa = 20; Table 1). Similarly, no variation was observed in several rodent species: *Akodon montensis* (2n = 24, FNa = 42), *Castoria angustidens* (2n = 46, FNa = 46), *Euryoryzomys russatus* (2n = 80, FNa = 86), *Juliomys ossitenuis* (2n = 20, FNa = 36), *J. pictipes* (2n = 36, FNa = 34), *Nectomys squamipes* (2n = 56; FNa = 56), *Oecomys catherinae* (2n = 60, FNa = 62) and *Thaptomys nigrita* (2n = 52, FNa = 52). We observed a karyotype of 2n = 40, FNa = 74 for the five squirrel specimens (*Guerlinguetus brasiliensis*) examined. The single specimen of *Rattus rattus* examined displayed a karyotype of 2n = 38, FNa = 58.

In contrast, intraspecific variation was observed in seven cricetid rodent species; six differed in fundamental number, with the seventh varying in both 2n and FNa (Table 1). All *Akodon cursor* specimens shared 2n = 14, although their fundamental numbers were 18 (*n* = 22), 19 (*n* = 19), 20 (*n* = 20) and 21 (*n* = 3; Table 1). Six *Brucepattersonius nebulosus* specimens (locality 21) displayed 2n = 52, FNa = 54/56 (Figure 2). Oryzomyine species exhibiting intraspecific variation included *Oligoryzomys nigripes*, which displayed a consistent diploid number (2n = 62) but varied in fundamental number (FNa), with one specimen displaying FNa = 81 and eight specimens displaying FNa = 82. Similarly, *Sooretamys angouya* showed variation, with two specimens having 2n = 58 and FNa = 60, and one having 2n = 58 and FNa = 61. Other species with karyotypic variation included *Rhipidomys itoan*, with seven specimens displaying 2n = 44 and FNa = 48, and three specimens displaying 2n = 44 and FNa = 50. For *Delomys dorsalis*, 12 specimens displayed 2n = 82 and FNa = 80, with four specimens displaying 2n = 82 and FNa = 82 (Table 1). All 14 specimens of *Trinomys dimidiatus* showed 2n = 60; of the seven specimens of this species

Table 1. List of species for which karyotypic data were obatined, including diploid chromosome number (2n) and fundamental autosomal number (FNa), sex pair (X and Y), locality number, and reference publications used for karyotype characterization. Legends: Locality numbers are described in the legend of Figure 1. Chromosome morphology: d = dot chromosome, la = large acrocentric, lm = large metacentric, lsm = large submetacentric, ma = medium acrocentric, mm = medium metacentric, msm = medium submetacentric, sa = short acrocentric, sm = short metacentric. Items in bold indicate new results or results that differ from the literature. An * in the references indicates results that differ from the present study.

TAXON	n	2n	FNa	X	Y	(Locality) Specimen number	Karyotype references
ORDER DIDELPHIMORPHIA							
Caluromys philander	1	14	**20**	sa	d	(20) MN84877	Souza *et al*. 2013
Gracilinanus microtarsus	2	14	20	**sm**	**d**	(20) RF64, RF80	Pereira and Geise 2007
Marmosops incanus	5	14	24	mm	sa	(2) MN79858; (20) MN83648, MN83649, MN83650, MN83651	Carvalho *et al.* 2002, Paresque *et al.* 2004, Di-Nizo *et al*. 2014
Monodelphis scalops	4	18	30	**sa**	**d**	(20) MN81902, MN81903, MN84921**,** MN84965	Di Nizo *et al*. 2014
Philander quica	1	22	20	sa	-	(20) MN84888	Carvalho *et al.* 2002, Faria *et al.* 2020
ORDER RODENTIA							
Family Cricetidae							
Tribe Akodontini							
Akodon cursor	22	14	18	sa	d	(4) MN85028, MN85034; (5) HGB-REGUA1, HGB-REGUA2, HGB REGUA17; (6) MN85013; (9) FI39; (13) MN85023, MN85025, MN84991, FS5-36, FS5-58, FS5-69, FS8-79; (14) MN76479, MN76487, MN76490, MN76497, MN76498, FS4-61; (16) MN48055; (17) MN85060	Fagundes *et al.* 1998, Geise *et al.* 1998, Faria *et al.* 2020
	19	14	19	sa	d	(1) MN83047; (3) MN85056, MN85057; (6) MN85016, MN85018, MN85019; (10) MN85007, MN85008; (12) FS5-59; (13) MN85024, FS5-3; (14) MN76494, MN76499**,** MN76474, MN76475, FS4-20, FS4-41, FS4-43, FS4-45	Fagundes *et al.* 1998, Geise *et al.* 1998, Faria *et al*. 2020
	19	14	20	sa	d	(4) MN85000, MN85029, MN85033, MN85037; (6) MN85014, MN85015; (7) FS6-13; (8) FS6-37; (10) MN85009, MN85010; (12) FS5-57; (13) MN85003; (14) MN76501, MN76476, FS4-6, FS4-25, FS4-31, FS4-33; (15) MN85053	Fagundes *et al.* 1998, Geise *et al.* 1998, Faria *et al*. 2020
	3	14	21	sa	d	(6) MN85017; (13) MN85020; (14) FS4-81	Fagundes *et al.* 1998, Geise *et al.* 1998, Faria *et al.* 2020
Akodon montensis	23	24	42	ma	sa	(18) MN31409; (19) MN59110; (20) MN84838, MN84839, MN84840, MN84843, MN84844, MN84847, MN84848, MN84852, MN84855, MN84858, MN84860, MN84870, MN84878, MN84879, MN84881, MN84885, MN84891; (21) MN84451, MN84460, MN84468; (25) EDH63	Fagundes *et al.* 1997*, Geise *et al.* 1998*, Fagundes *et al*. 2000
	1	24	42	ma	sm	(25) JDM2	Fagundes *et al.* 1997*, Geise *et al.* 1998*, Fagundes *et al*. 2000
Brucepattersonius nebulosus	4	52	**54**	la	sa	(21) MN84453, MN84452, MN84466, MN84467	Present study
	2	52	**56**	msm	sm	(21) MN84464, MN84465	Present study
	1	52	-	-	-	(20) MN84884	-
Castoria angustidens	12	46	46	ma	sa	(21) MN84454, MN84455, MN84456, MN84462, MN84463, MN84461, MN84469; (24) MN69812; (24) MN77078, MN77079, MN77084, MN77097	Christoff *et al*. 2000*, Abreu *et al*. 2014*, Pardiñas *et al*. 2015*, 2016*
	3	46	-	-	-	(24) MN69807, MN77099, MN77117	-
Thaptomys nigrita	3	52	52	ma	**sm**	(19) MN69838; (20) MN84883, MN84893	Yonenaga 1975, Faria *et al*. 2020*
Oxymycterus sp.	1	54	62	**la**	sa	(23) MN48063	Svartman and Cardoso de Almeida 1993, Oliveira and Gonçalves 2015*
Tribe Oryzomyini							
Euryoryzomys russatus	2	80	86	lsm	sa	(15) MN71797, MN71798	Di-Nizo *et al.* 2014
	1	80	-	-	-	(15) MN48023	-
Nectomys squamipes	1	56	56	lsm	**sa**	(10) MN67049	Silva and Yonenaga-Yassuda 1998
Oecomys catherinae	1	60	62	lsm	la	(11) MFD1	Malcher *et al.* 2017
	1	60	-	-	-	(10) MN74373	-
Oligoryzomys nigripes	1	62	81	lsm	sa	(20) RF1411	Faria *et al.* 2020
	6	62	82	lsm	**ma**	(1) MN83048; (20) MN81916, MN81917, MN81938, MN84892, MN84912	Faria *et al.* 2020
	2	62	82	lm	**ma**	(20) MN81921, MN84913	Faria *et al.* 2020
	3	62	-	-	-	(5) HGB-REGUA14; (19) MN67472; (17) MN69888	-
Sooretamys angouya	2	58	60	la	-	(20) MN84966	Di-Nizo *et al.* 2014, Faria *et al*. 2020
	1	58	**61**	la	-	(20) MN84970	Di-Nizo *et al.* 2014, Faria *et al*. 2020
Tribe Thomasomyini							
Rhipidomys itoan	7	44	48	lsm	sa	(16) MN46801, MN46805, MN63626, MN63016, MN63605; (20) MN81934, MN81935	Pinheiro and Geise 2008*, Costa *et al.* 2011
	3	44	50	lsm	**ma**	(15) HGB398; (20) MN81932, MN84923	Pinheiro and Geise 2008*, Costa *et al.* 2011
Tribe Wiedomyini							
Juliomys ossitenuis	1	20	36	**lm**	**sa**	(19) MN81077, MN81078; (20) MN81079, MN81080, MN81081, MN81082, MN81083, MN81084, MN81085, MN81086, MN81087, MN81088, MN81089, MN81090, MN81091, MN81092; (21) MN84458	Aguieiras *et al.* 2013, Souza *et al.* 2020
Juliomys pictipes	4	36	34	ma	sa	(11) MFD2; (16) MN81095, MN81096; (20) MN81097	Bonvicino and Otazu 1999, Di-Nizo *et al.* 2014
	1	36	-	-	-	(22) MN81094	-
Incertae Sedis							

Delomys dorsalis	13	82	80	lm	sa	(20) MN84837, MN84841, MN84845, MN84850, MN84851, MN84853, MN84859, MN84861, MN84863, MN84864, MN84865, MN84866; (21) MN84459	Gonçalves and Oliveira 2014*
	3	82	82	lm	sa	(20) MN84836, MN84857, MN84862	Present study
						Family Echimyidae	
Trinomys dimidiatus	6	60	112	lsm	**sa**	(19) MN67503; (20) MN81087, MN84842, MN84849, MN84854, MN84867	Present study
	1	60	114	lsm	**sa**	(10) MN67511	Delciellos *et al.* 2023
	7	60	-	-	-	(19) MN67504; (20) MN84869, MN84871, MN84872, MN84873, MN84874, MN84875	-
						Tribe Echimyini	
Phyllomys pattoni	3	**76**	**128**	**lm**	**sa**	(16) DL19, DL20, DL21	Present study
	1	**76**	-	-	-	(10) MN42978	Present study
Phyllomys sp.	1	**52**	**96**	**lm**	**d**	(6) MN84016	Present study
						Family Muridae	
Rattus rattus	1	38	58	la	sa	(20) MN84962	Kasahara and Yonenaga-Yassuda 1981
						Family Sciuridae	
Guerlinguetus brasiliensis	4	40	74	lsm	ma	(19) MN69839; (16) MN69865; (20) MN81904, MN81907	Fagundes *et al.* 2003
	1	40	-	-	-	(20) MN81906	-

that allowed definition of the FNa, six had FNa = 112 and one FNa = 114.

All four specimens of *Phyllomys pattoni* exhibited 2n = 76. Three of these specimens displayed FNa = 128 (Table 1; Figure 3); this represents the first karyotypic data for this species. Additionally, one specimen (MN84016) identified as *Phyllomys* sp. by Araújo *et al*. (2014; = *Phyllomys* sp. 3) displayed a previously undescribed karyotype of 2n = 52 and FNa = 96 (Figure 4; Table 1).

Discussion

In recent years, the importance of cytogenetic analyses in understanding the biodiversity and evolution of South American small mammals has become increasingly evident. Our study focuses on the non-volant small mammals of the Serra dos Órgãos and surrounding regions, with particular emphasis on karyotypic analysis of specimens from the protected area of PARNASO. Although trapping efforts across the altitudinal range of this area were not equivalent due to varying research goals and logistical constraints, our cytogenetic data have provided crucial insights into the taxonomy and identification of these mammals. This is especially true for the sigmodontine rodents, which present challenges in field identification due to their cryptic nature. Specimens analyzed here were either collected by other researchers who had various non-genetic goals (*e. g.*, Delciellos *et al.* 2016, 2017, 2019) or were collected according to the geographic characteristics of the region (access, possibility of trapping) and available logistical infrastructure. In general, the purpose of the associated cytogenetic analyses was to assist in the identification of species as part of ecological studies. The collections made in this way reflect the taxonomic challenges noted here - sigmodontine rodents present greater difficulty for identification at the specific level in the field and thus more specimens of this subfamily were collected for identification using karyotypes and other techniques. In the study area, marsupials can be easily identified in the field by their external morphological characteristics. The sampled area has been extensively studied since the 1990s, with long-term monthly collections. As a result, the marsupial fauna is well-known to the point that species can be named based on morphology with a high degree of certainty. Other areas may have marsupials whose species are still under review, which could lead to taxonomic uncertainties. However, the

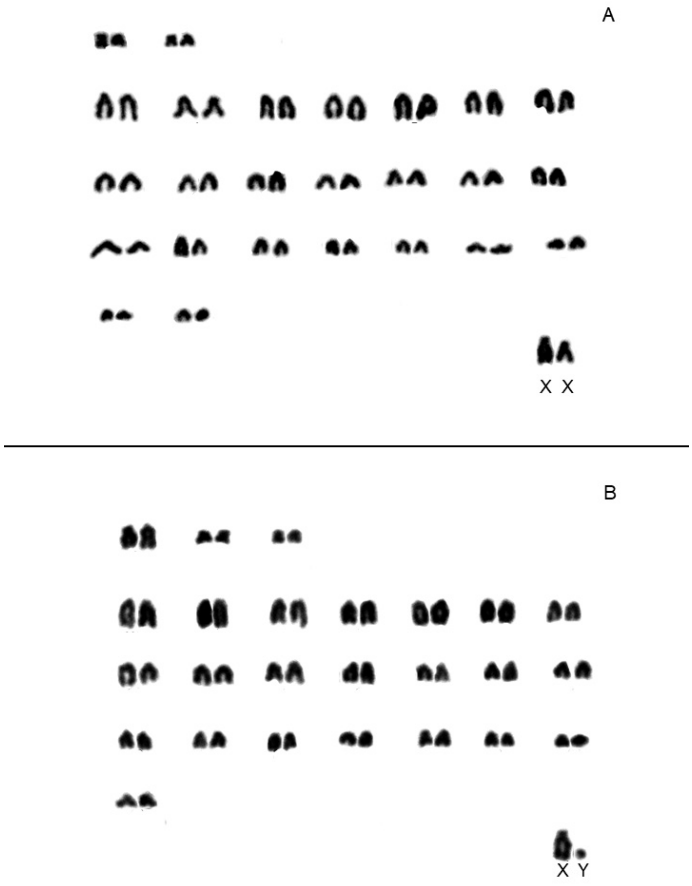

Figure 2. Karyotype of *Brucepattersonius nebulosus* specimens (A = MN84466) and B = MN84465) obtained using conventional staining. 2n = 52, FNa = 54 and 2n = 52, FNa = 56, respectively.

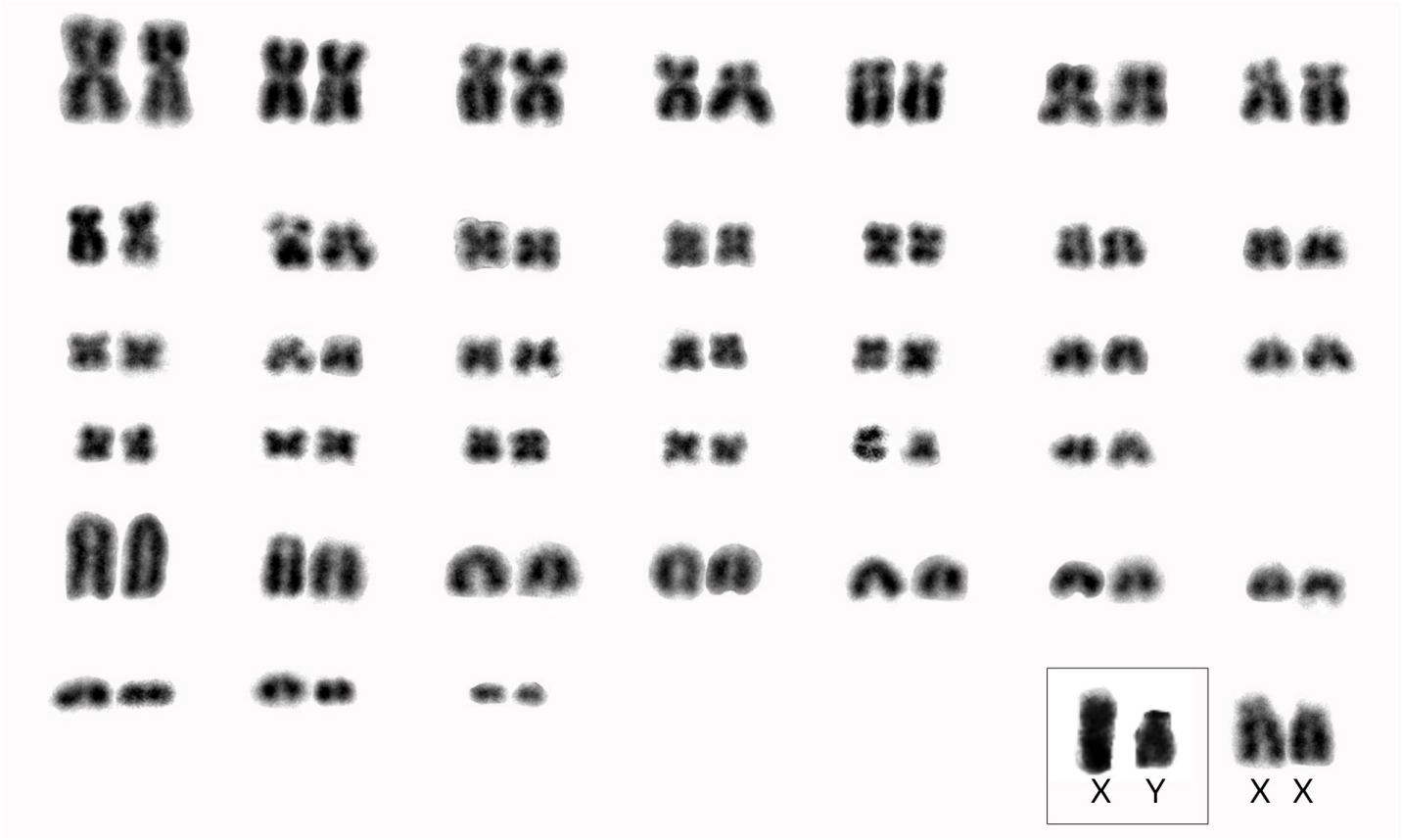

Figure 3. Karyotype a of a *Phyllomys pattoni* specimen (DL19, female) obtained using conventional staining, 2n = 76, FNa = 128. Sex chromosomes from a male are from DL21.

species in Serra dos Órgãos appear to be well established without significant taxonomic doubts— which is why we have had few specimens of this order for cytogenetic studies (Supplementary material).

Another important point that must be highlighted is the interpretation of the morphology of the chromosomes. We consider the fundamental number of *Monodelphis scalops* to be the same as that in Di-Nizo *et al.* (2014), which is in accord with Levan *et al*. (1964) but contrary to Faria *et al.* (2020), who consider the FNa to be 30. We consider the fundamental number for *Gradilinanus microtarsus* to be 20, which differs from Pereira *et al.* (2008), who considered this number to be 24.

According to Abreu *et al.* (2021), *Brucepattersonius nebulosus* is the only species in that genus with a FNa differing from that reported by Bonvicino *et al.* (1998), who found 2n = 52, FNa = 53 in two specimens collected in Itamonte (MG) due to the presence of heteromorphic pairs. Based on the karyotype observed by us for the Serra dos Órgãos sample (2n = 52, FNa = 54/56; locality 21), we confirm that the karyotype of *B. nebulosus* differs from all other species of the genus (*B. griserufescens*, *B. soricinus* and *B. iheringi*). A different result (2n = 52; FNa = 52) was also found in one specimen collected in the Parque Nacional da Bocaina (Delciellos *et al*. 2023), which is close to the type locality of *B. nebulosus* (Abreu-Júnior and Percequillo 2019). Similarly, the variation in the fundamental number described here for *Delomys dorsalis* (2n = 82 and FNa = 82) adds a new variant to this species, since the only previously known karyotype is 2n = 80 and FNa = 82 (Di-Nizo *et al.* 2017). We recognize the need for a more detailed chromosomal banding analysis (*e. g*., Malcher *et al.* 2017) to gain a clearer and more comprehensive understanding of the chromosomal forms of these taxa and we suggest that data on banding patterns will clarify the chromosomal evolution of these animals (Figure 2).

The high variation in the fundamental number for *Trinomys dimidiatus* described here is highlighted in Table 1. Banding pattern techniques are also necessary to understand this variation, which may reflect the presence of B chromosomes as suggested by Fagundes *et al.* (2004) for *T. iheringi*. Nacif *et al.* (2023) demonstrated the high taxonomic diversity within this genus, proposing different lineages that probably represent undescribed species, in the southeastern portion of the Brazilian Atlantic Forest.

The genus *Phyllomys*, one of the most intriguing among echimyid rodents (Araújo *et al.* 2014), was intensively studied by Leite (2003), who documented the occurrence of more than one species in the Serra dos Órgãos region. Our sample comprises specimens exclusively recorded in the lowest elevations of the area under study (localities 6, 10, and 16, Figure 1), previously identified through DNA analysis (Araújo *et al.* 2014; Delciellos *et al.* 2017).

Our four karyotyped specimens of *Phyllomys pattoni* revealed an undescribed diploid number (2n = 76), with three specimens allowing the determination of a fun-

damental number of autosomes (FNa) = 128 (Table 1). Emmons *et al.* (2002) cited a karyotype for *P. pattoni* (2n = 80, FNa = 112) from a specimen trapped in Espírito Santo state and reported a karyotype of 2n = 72, FNa = 114 for one of the specimens reported here (MN42978, Table 1) via personal communication to one of the authors of this article (L. Geise). However, this information is incorrect, as our extended analysis of this specimen's karyotype could not define the FNa. Moreover, the correct 2n for this specimen is 76, the same found in the three specimens we karyotyped from locality 16, where analysis determined a fundamental number of 128 (Figure 3, Table 1).

Our results serve to correct information provided by Emmons *et al.* (2002). Our species identifications are robust, having been confirmed by molecular analysis that included samples from various localities and karyotypic forms of this species. It is important to consider that *P. pattoni* includes individuals with high chromosomal variation, with 2n ranging from 72 to 80 and FNa ranging from 110 to 128, similar to what has been observed in *P. nigrispinus* (Paresque *et al.* 2004; Delciellos *et al.* 2017). Clearly, further studies are needed to clarify the nature and significance of this extensive geographical variation.

Loss and Leite (2011), Araújo *et al.* (2014), and Abreu-Júnior *et al*. (2018) identified an undescribed taxon (*Phyllomys* sp. 3) through molecular analyses. We karyotyped one of the individuals that they examine (MN84016, previously cited by these authors by its field number, FS12-30), revealing a new karyotype for the genus (Table 1). This important finding adds a new diagnostic character for the accurate identification of *Phyllomys* species, further emphasizing the utility of karyotyping all Atlantic spiny tree rat specimens collected throughout the distribution of the genus. The high morphological (Leite and Patton 2002) and genetic variation observed in this genus needs to be studied in greater detail to better understand the complex evolutionary and biogeographic patterns of South American small mammals.

Cronemberger *et al.* (2019) provided a list of 45 species of non-volant small mammals (13 marsupials and 32 rodents) recorded in PARNASO. In this study, we present karyotypic data for five marsupials and 20 rodents collected in PARNASO and surrounding areas, covering over half of the species in this protected area. These karyotypic data have facilitated better identification of cryptic species and the description of new taxa. We also emphasize that a correct interpretation of chromosomal morphology is essential for making comparisons between samples, thereby enhancing our understanding of the variation observed among species and across localities.

In conclusion, our study highlights the significant role of cytogenetic analyses in enhancing taxonomic resolution and understanding of biodiversity among South American small mammals. By focusing on the karyotypic diversity within the Serra dos Órgãos region and its surroundings, we have elucidated chromosomal variations and taxonomic complexity within these species. Our findings underscore the importance of integrating different types

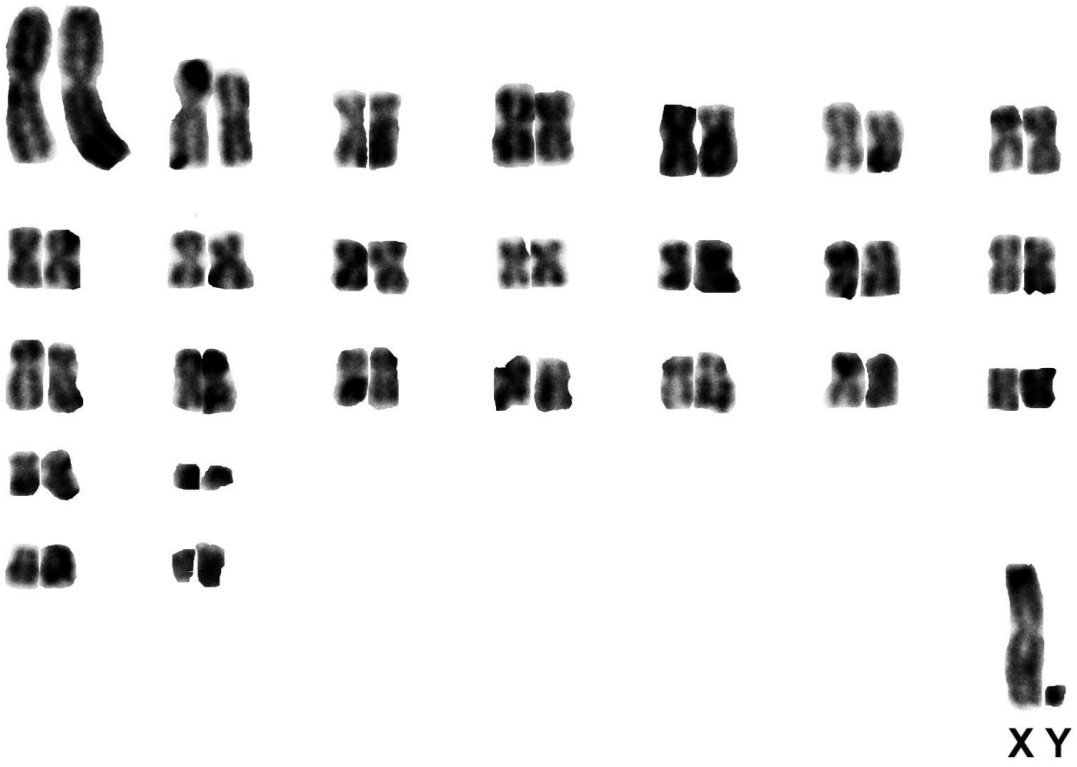

Figure 4. Karyotype of *Phyllomys* sp. (MN84016) obtained using conventional staining, 2n = 52, FNa = 96.

of data to identify cryptic species and to refine our understanding of the evolutionary and biogeographic dynamics that have shaped the fauna of the Brazilian Atlantic Forest. Continued research utilizing comprehensive cytogenetic and molecular techniques will be essential for uncovering the hidden diversity and evolutionary relationships within this ecologically significant region, ultimately contributing to more informed conservation strategies for the region's unique biodiversity.

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Supplementary material

List of specimens for each species of Didelphimorphia and Rodentia (in alphabetic order) analyzed in this study. Numbers in parenthesis correspond to localities provided in the legend of Figure 1. MN correspond to Museu Nacional voucher numbers; Field numbers for specimens are: FS and RF - LabVert, HGB-REGUA - H.G. Bergallo, LG – L. Geise, EDH - Erika Hingst-Zaher, DL - Diogo Loretto, and MFD – M.F. Dalloz.

DIDELPHIMORPHIA

Caluromys philander: (20) Male: MN84877

Gracilinanus microtarsus: (20) Female: RF80. Male: RF64

Marmosops incanus: (2) Male: MN79858; (20) Females: MN83650, MN83651. Males: MN83648, MN83649

Monodelphis scalops: (20) Female: MN81902, MN84921. Males: MN81903, MN84965

Philander quica: (20) Female: MN84888

RODENTIA

Family Cricetidae

Akodon cursor: (1) Male: MN83047; (3) Females: MN85056, MN85057; (4) Females: MN85000, MN85028, MN85034, MN85037. Males: MN85029, MN85033; (5) Females: HGB-REGUA1, HGB-REGUA17. Male: HGB-REGUA2; (6) Females: MN85013, MN85015, MN85017. Males: MN85014, MN85016, MN85018, MN85019; (7) Male: FS6-13; (8) Male: FS6-37; (9) Male: FI39; (10) Females: MN85009. Males: MN85007, MN85008, MN85010; (12) Males: FS5-57, FS5-59; (13) Females: MN84991, FS5-36, MN85003, MN85020, MN85023, MN85025. Males: FS5-3, FS5-58, FS5-69, FS8-79, MN85024. (14) Females: MN76474, FS4-20, FS4-31, FS4-33, MN76487, FS4-41, MN76494, MN76497. Males: MN76475, MN76476, MN76479, FS4-06, FS4-25, MN76490, FS4-43, FS4-45, MN76498, MN76499, MN76501, FS4-61, FS4-81; (15) Female: MN85053; (16) Female: MN48055; (17) Male: MN85060.

Akodon montensis: (18) Female: MN31409; (19) Males: MN59110; (20) Females: MN84839, MN84843, MN84852, MN84860, MN84879. Males: MN84838, MN84840, MN84844, MN84847, MN84848, MN84855, MN84858, MN84870, MN84878, MN84881, MN84885, MN84891; (21) Males: MN84451, MN84460, MN84468; (25) Female: EDH63. Male: JDM2.

Brucepattersonius nebulosus: (20) Male: MN84884; (21) Females: MN84452, MN84453, MN84466, MN84467. Males: MN84464, MN84465.

Castoria angustidens: (21) Females: MN84454, MN84463, MN84469. Males: MN84455, MN84456, MN84461, MN84462; (24) Females: MN69807, MN69812, MN77117. Males: MN77099; Indeterminate: MN77078, MN77079, MN77084, MN77097

Delomys dorsalis: (20) Females: MN84836, MN84841, MN84845, MN84851, MN84859, MN84846, MN84862, MN84865. Males: MN84837, MN84850, MN84853, MN84857, MN84861, MN84863, MN84866; (21) Male: MN84459.

Euryoryzomys russatus: (15) Females: MN71798, MN48023. Male: MN71797.

Juliomys ossitenuis: (19) Female: MN81077. Male: MN81078; (20) Females: MN81083, MN81084, MN81088, MN81092. Males: MN81079, MN81080, MN81081, MN81082, MN81085, MN81086, MN81087, MN81089, MN81090, MN81091; (21) Male: MN84458.

Juliomys pictipes: (11) Male: MFD2; (16) Males: MN81095, MN81096; (20) Female: MN81097; (22) Male: MN81094.

Nectomys squamipes: (10) Males: MN67049.

Oecomys catherinae: (10) Males: MN74373; (11) Males: MFD1.

Oligoryzomys nigripes: (1) Male: MN83048; (5) Female: HGB-REGUA14; (19) Female: MN67472; (20) Female: MN81917. Males: MN84892, MN81916, MN81921, MN81938, MN84912, MN84913, RF1411; (17) Male: MN69888.

Oxymycterus sp: (23) Male: MN48063.

Rhipidomys itoan: (15) Female: HGB 398; (16) Females: MN46805, MN63626, MN63016. Males: MN46801, MN63605; (20) Females: MN81934, MN81935. Males: MN81932, MN84923.

Sooretamys angouya: (20) Females: MN84966, MN84970.

Thaptomys nigrita: (19) Female: MN69838; (20) Males: MN84883, MN84893.

Family Echimyidae

Phyllomys pattoni: (10) Female: MN42978; (16); Females: DL19, DL20. Male: DL21.

Phyllomys sp.: (6) Males: MN84016.

Trinomys dimidiatus: (10) Male: MN67511; (19) Males: MN67503, MN67504; (20) Females: MN84842, MN84849, MN84854, MN84867, MN84871. Males: MN84869, MN84872, MN84873, MN84876, MN84875, MN81087.

Family Muridae

Rattus rattus: (20) Male: MN84962.

Family Sciuridae

Guerlinguetus brasiliensis: (19) Male: MN69839; (16) Female: MN69865; (20) Females: MN81906, MN81907. Male: MN81904.

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Are the Heteromyidae paraphyletic? Molecular phylogenetics of extant geomyoid rodents

Enrique P. Lessa*, and Andrés Parada

Departamento de Ecología y Evolución, Facultad de Ciencias, Universidad de la República. Iguá 4225, Montevideo 11400, Uruguay. E-mail: enrique.lessa@gmail.com (EPL); andrespara@gmail.com (AP).
*Corresponding author: https://orcid.org/0000-0001-9750-4093.

The superfamily Geomyoidea includes the Geomyidae (pocket gophers: 7 genera, 42 species) and Heteromyidae (pocket mice, kangaroo mice, and kangaroo rats: 5 genera, 69 species). Analyses of both morphological and molecular data have confirmed the monophyly of the superfamily relative to other rodents but have cast doubts on the reciprocal monophyly of Geomyidae and Heteromyidae. The latter are recovered as paraphyletic in some, but not all molecular phylogenies, with low to moderate support for critical nodes. To test alternative hypotheses of geomyoid phylogenetic relationships, we searched NCBI databases and assembled four datasets: a) the 13 protein-coding mitochondrial DNA genes (7 genera, 13 species); b) the mitochondrial 12s, 16s, and COX1 loci (7 genera, 43 species); and two datasets of ultraconserved elements (UCEs; 6 genera, 9 species): c) one with 3,991 loci, allowing for up to 2 unrepresented taxa per locus, and d) another one reduced to 1,750 UCEs (with no missing data). In all cases, beavers were included as outgroups. Maximum likelihood analysis of both mitochondrial datasets were equivocal regarding heteromyid paraphyly, as support for the critical nodes was very low. In contrast, the trees obtained from UCE loci with both Maximum Likelihood and a multi-species coalescent method (wASTRAL) indicated that pocket gophers were sister to pocket mice (100 % bootstrap, local Posterior Probability of 1), to the exclusion of kangaroo rats, which formed a second, strongly supported clade (100 % bootstrap, local Posterior Probability of 1; Figure 3). These preliminary findings are consistent with the hypothesis that Heteromyidae are paraphyletic relative to Geomyidae. The inclusion of additional taxa (*e. g.*, kangaroo mice) in the analyses is required to confirm these results.

La superfamilia Geomyoidea incluye Geomyidae (tuzas: 7 géneros, 42 especies) y Heteromyidae (ratones de abazones, ratones y ratas canguro: 5 géneros, 69 especies). Los análisis de datos tanto morfológicos como moleculares han confirmado la monofilia de la superfamilia con respecto a otros roedores, pero arrojaron dudas sobre la monofilia recíproca de Geomyidae y Heteromyidae. Estos últimos se recuperan como parafiléticos en algunas, pero no en todas, las filogenias moleculares, con un apoyo bajo a moderado para los nodos críticos. Para poner a prueba hipótesis alternativas sobre las relaciones filogenéticas de los geomioideos, buscamos en las bases de datos del NCBI y reunimos cuatro dos conjuntos de datos: a) los 13 genes del ADN mitocondrial que codifican proteínas (7 géneros, 13 especies); b) los loci mitocondriales 12s, 16s y COX1 (7 géneros, 43 especies); y dos conjuntos de datos de elementos ultraconservados (UCEs; 6 géneros, 9 especies): c) uno con 3,991 loci, permitiendo hasta 2 taxa no representados por locus; y d) otro con 1,750 UCEs (sin datos faltantes). En todos los casos, los castores fueron incluidos como grupo externo. El análisis de Máxima Verosimilitud de loci mitocondriales concatenados recuperó a Geomyidae y Heteromyidae como taxones hermanos, pero el apoyo de "bootstrap" para la monofilia de este último fue sólo del 55 %. Por el contrario, los árboles obtenidos de loci UCE, tanto con Máxima Verosimilitud como con un método coalescente para múltiples especies (wASTRAL) indicaron que las tuzas eran hermanas de los ratones de abazones (valor de "bootstrap" de 100%, Probabilidad Posterior local de 1), con exclusión de las ratas canguro, que formaban un segundo clado fuertemente apoyado (valor de "bootstrap" de 100%, Probabilidad Posterior local de 1). Estos hallazgos preliminares son consistentes con la hipótesis de que la familia Heteromyidae es parafilética con respecto a Geomyidae. Se requiere la inclusión de taxones adicionales (*e. g.*, ratones canguro) en los análisis para confirmar estos resultados.

Keywords: Geomyidae; Geomyoidea; mitogenomes; systematics; ultraconserved elements.

Introduction

Our understanding of the diversity and phylogenetic relationships of rodents has been greatly impacted by DNA sequencing data. Molecular phylogenetic studies have helped support alternative hypotheses based on morphological and paleontological data, while also suggesting new phylogenetic relationships and uncovering hidden diversity among morphologically cryptic species. The integration of molecular and morphological data has resulted in the recognition of three major clades of extant rodents, namely the squirrel-related clade (suborder Sciuromorpha), the guinea pig-related clade (suborder Hystricomorpha = Ctenohystrica), and the mouse-related clade (reviewed by Fabre *et al.* 2015; D'Elía *et al.* 2019; see also Swanson *et al.* 2019; Bangs and Steppan 2022).

The mouse-related clade includes three major groups, typically recognized as suborders: 1) the families Anomaluridae, Pedetidae, and Zenkerellidae (Suborder Anomaluromorpha); 2) a diverse assemblage of nine families, grouped into the superfamilies Muroidea and Dipodoidea (Suborder Myomorpha; and 3) a clade including the families Castoridae, Heteromyidae, and Geomyidae (Suborder Castorimorpha). These three suborders may be treated as infraorders if the mouse-related clade is recognized as the Suborder Supramyomorpha (D'Elía *et al.* 2019; Flynn *et al.* 2019).

Here, we focus on the phylogenetic relationships of extant Castorimorpha (or Castorimorphi), an assemblage of some 113 species comprising the Castoridae (2 species of beavers), the Geomyidae (42 species of pocket gophers), and the Heteromyidae, subdivided into the subfamilies Heteromyinae (17 species of spiny pocket mice), Perognathinae (30 species of pocket mice), and Dipodomyinae (2 species of kangaroo mice and 20 species of kangaroo rat, (Table 1; Mammal Diversity Database 2023). A. E. Wood (1955) first proposed the suborder Castorimorpha to include the Castoridae and the fossil family Eutypomidae. An unanticipated result of molecular phylogenetic studies was the sister taxon relationship between Castoridae and the superfamily Geomyoidea Weber 1904 (*cf*. Wahlert 1985), that includes the families Heteromyidae and Geomyidae (Huchon *et al.* 1999; Murphy *et al.* 2001; Adkins *et al*. 2003; DeBry 2003; but see DeBry and Sagel 2001). Carleton and Musser (2005) tentatively accepted the inclusion of Geomyidae and Heteromyidae within Castorimorpha, an arrangement that was subsequently supported by additional molecular phylogenetic studies (*e. g.* Montgelard *et al.* 2008; Blanga-Kanfi *et al.* 2009; Fabre *et al.* 2012; Fabre *et al.* 2015; Swanson *et al.* 2019; but see Veniaminova *et al.* 2007).

Recognition of the close relationships of Geomyidae and Heteromyidae (superfamily Geomyoidea) predates their inclusion in the Castorimorpha (*e. g.* Wood 1931) and has received strong, confirmatory support from molecular phylogenetics (reviewed by Fabre *et al.* 2015). Both families share the following characteristics: "shortened incisive foramina (less than half diastema length); posterior maxillary foramen closed; cranially, the premaxillae extend further posteriorly than the nasal bones; there is a parapterygoid fossa and a bulla at the back of foramen ovale; the cheek teeth tend to be high crowned and lophodont, with stylar cusps well developed" (Flynn *et al.* 2008). The reciprocal monophyly of Geomyidae and Heteromyidae, however, has been questioned on both morphological and molecular grounds. In particular, paleontological studies relying on similarity in dental morphology have suggested that both extant pocket gophers and heteromyids might have been derived from ancestors similar to pocket mice that may be placed within the Heteromyidae (*e. g.*, Lindsay 1972; Korth (1994).

However, Wahlert (1985: pp. 14 to 15) examined the cranial morphology of both extant and fossil geomyoids and identified 18 synapomorphies in support of the monophyly of pocket gophers, as well as seven synapomorphies in support of the monophyly of extant heteromyids. Analyses of myological characters also identified several synapomorphies in support of the reciprocal monophyly of Geomyidae and Heteromyidae (Ryan 1989). In contrast, Brylski (1990) examined the development and adult anatomy of carotid circulation of geomyoids and found that both pocket gophers and *Heteromys* lost the stapedial arteries in adults, in contrast with other heteromyids. If the absence of stapedial arteries found in pocket gophers and *Heteromys* is a derived condition, it suggests either heteromyid paraphyly or parallel evolution of carotid circulation in these taxa (Brylski 1990). In general, morphological analyses of relationships among extant geomyoids have favored reciprocal monophyly of at least crown Heteromyidae and Geomyidae (see discussion in Wahlert 1985; Korth (1994; Hafner *et al.* 2007).

Early molecular phylogenetic analyses of higher-level relationships among rodents (*e. g.* (Huchon *et al.* 1999; Adkins *et al.* 2001; Adkins *et al*. 2003) typically included few castorimorphs. DeBry (2003) included three geomyoids in his analysis of rodent phylogenetics and obtained moderate support for a *Thomomys* + *Chaetodipus* clade to the exclusion of *Dipodomys*. In contrast, Montgelard *et al.* (2008) recovered Geomyidae (represented by three genera) and Heteromyidae (represented by *Dipodomys* and *Heteromys*) as reciprocally monophyletic.

Using sequence data from three mitochondrial loci (12s ribosomal RNA, 16s ribosomal RNA, and COX1), Hafner *et al.* (2007) examined phylogenetic relationships within the Heteromyidae, with multiple species per genus and four genera of Geomyini as outgroup taxa. This study offered moderate to strong support for each of the three heteromyid subfamilies (Figure 1). Hafner *et al.* (2007) also reviewed the relevant literature and argued in favor of the reciprocal monophyly of Heteromyidae and Geomyidae, although no outgroup to the Geomyoidea was included in their study.

The analysis of Fabre *et al.* (2012) included a total of 87 species of castorimorphs (31 geomyids, 54 heteromyids, and the 2 extant beaver species), representing a major advance in taxonomic representation in a multilocus analysis of higher-level relationships among rodents. Fabre *et al.* (2012) analyzed DNA sequence data from six mitochondrial and five nuclear loci, recovering both the castorimorph clade and the sister-taxon relationships of Castoridae and Geomyoidea. However, Heteromyidae were recovered as paraphyletic relative to Geomyidae and, within heteromyids, the Perognathinae were not recovered as monophyletic (Figure 1).

Although Fabre *et al.* (2012) used a total of 11 loci, these were unevenly represented across taxa. The number of mitochondrial loci represented in at least one species per genus ranged from 1 to 4, whereas the corresponding range for nuclear loci was 0 to 5. Furthermore, the nodes that are key to the paraphyly of heteromyids relative to geomyids were not well supported (Figure 1). In their review of rodent phylogenetics, Fabre *et al.* (2015) combined the loci used by Montgelard *et al.* (2008) and Blanga-Kanfi et al. (2009); in the resulting phylogeny (their Figure 2.1), *Dipodomys* was recovered as sister to three geomyid genera, forming a clade that excluded *Heteromys*, but this relationship was weakly supported.

Within the Geomyidae, molecular phylogenetic analyses strongly support the classical view that the tribes Thomomyini (genus *Thomomys*) and Geomyini (six gen-

era, see Table 1) are sister taxa (Spradling *et al.* 2004; Fabre *et al.* 2012).

Recently, datasets with numerous nuclear loci have enabled some authors to revisit these phylogenetic hypotheses. A study based on 2,213 ultraconserved elements (UCEs, Swanson *et al.* 2019) found high support for both castorimorphs and geomyoids, but the relationships within the latter (represented by one pocket gopher, one kangaroo rat, and one pocket mouse species) were unresolved. Upham *et al.* (2019) used a "backbone and patch" approach to examine available DNA sequence data to build a phylogeny of all extant mammalian species. Their study included sequence data for 109 castorimorphs, represented by up to 24 nuclear and three mitochondrial loci. Their phylogeny *(e. g.* Upham *et al.* 2019: Supplementary Material 3 Data) also shows Heteromyidae as paraphyletic, but pocket gophers appear as sister to pocket mice, a result different from that of Fabre *et al.* (2012). Again, support for heteromyid paraphyly is moderate (91 % bootstrap support; Figure 1). Table 2 provides a summary of the taxonomic and genetic coverage of selected molecular phylogenetic studies.

In summary: 1) the suborder Castorimorpha (or infraorder Castorimorphi) has been strongly supported by molecular data and includes the family Castoridae and the Geomyoidea as a strongly supported superfamily; 2) among the latter, the Geomyidae have consistently been recovered as a monophyletic group; 3) some, but not all morphological and, especially, several molecular phylogenetic analyses suggest that Heteromyidae are paraphyletic relative to Geomyidae; however, 4) trees that suggest paraphyly are not consistent in the identity of the closest relatives of Geomyidae among Heteromyidae, and support for the key nodes is low to moderate in these phylogenies.

Recent genomic projects, including Zoonomia (https://zoonomiaproject.org/) and the California Conservation Genomics Project (https://www.ccgproject.org/) have made available high quality genomes of several castorimorphs. Additionally, the annotation of vertebrate mitogenomes from raw sequences has been greatly simplified by new software (*e. g.* Meng *et al.* 2019), making it possible to improve the representation for mitochondrial sequences. In this study, we take advantage of full genomes and other genomic datasets to revisit the issue of phylogenetic relationships of families and subfamilies within the Geomyoidea, with the goal of reassessing the hypothesis of heteromyid paraphyly. To this end, we use two main types of genomic data: a mitogenomic dataset that includes complete codons of the 13 protein-coding genes encoded in the mitochondrial genome; and a dataset of UCEs that combines the loci sequenced by Swanson *et al.* (2019) with their orthologs obtained by mining five additional available castorimorph genomes. We also reanalyzed the data from Hafner *et al.* (2007) after adding sequences of the two extant beaver species (*Castor canadensis* and *C. fiber*) as outgroups.

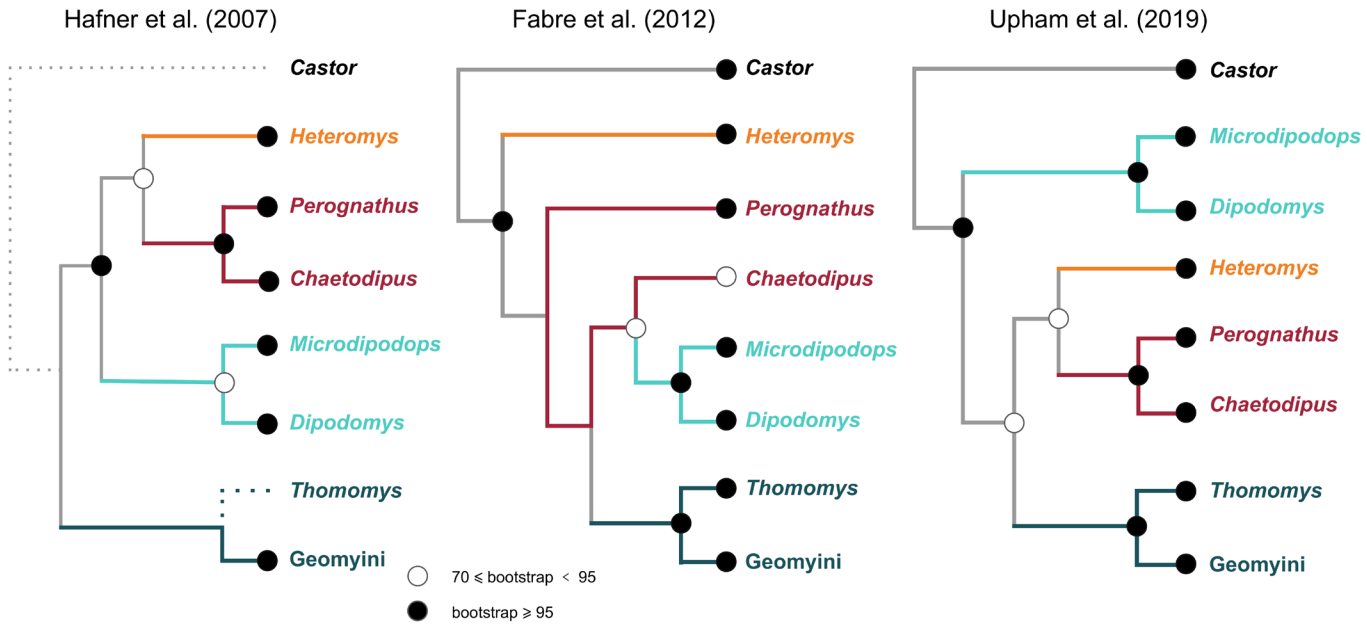

Figure 1. Three hypotheses of phylogenetic relationships among castorimorphs. The currently recognized subfamilies of Heteromyidae are the Heteromyinae (orange), Perognathinae (maroon) and Dipodomyinae (turquoise). Circles denote support for the corresponding nodes in the trees. Hafner *et al.* (2007) argue for reciprocal monophyly of Geomyidae and Heteromyidae; this study did not include *Castor* or *Thomomys*, but their positions in the tree (dotted lines) are implied in the author's discussion. Fabre *et al.* (2012) recovered Heteromyidae as paraphyletic relative to Geomyidae, with the latter being sister to an assemblage of Dipodomyinae + *Chaetodipus*. Upham *et al.* (2019) recovered Heteromyid paraphyly, with Geomyidae sister to pocket mice (Heteromyinae + Perognathinae).

Materials and methods

Mitogenomes. The protein-coding sequences of annotated mitogenomes were downloaded from NCBI for the following species: *Castor canadensis* (NC_033912.1; Lok *et al*. 2017); *Castor fiber birulai* (OQ731787.1); *Chaetodipus penicillatus* (NC_068810.1); *Dipodomys spectabilis* (NC_068808.1); *Dipodomys merriami* (NC_068807.1); *Geomys pinetis* (NC_069016.1); and *Thomomys bottae* (CM063115.1). Additional sets of protein-coding loci encoded by the mitochondrial genome were assembled de novo from RNAseq or genomic reads available in the Sequence Read Archive (SRA) of NCBI: *Perognathus longimembris* (SRR1846776; Kozak *et al.* 2024); *Chaetodipus baileyi* (SRR1633402); *Chaetodipus intermedius* (SRR12430174); *Heteromys desmarestianus* (SRR12430176); *Dipodomys ordii* (SRR1646418); and *Dipodomys stephensi* (SRR14572526). In these cases, mitogenomes were obtained with the software MitoZ 3.6 (Meng *et al.* 2019). The final dataset included the 13 protein-coding loci for 13 taxa, with the following missing data: MT-ND1 (Mitochondrially Encoded NADH Dehydrogenase 1 was not recovered for the two species of beavers and *Chaetodipus intermedius*, and MT-ND2 was not recovered for the two species of beavers, *C. intermedius*, and *Perognathus longimembris*.

Not all mitogenomic data are linked to museum vouchers. As a check of the species identity of the sequences included in our study, we used BLAST (Camacho *et al*. 2009) on the NCBI site to assess the similarity of the CYTB and COX1 DNA sequences of each of our taxa against the NCBI Nucleotide database. In all cases, the highest similarity scores were to sequences of the same named species reported by a peer-reviewed publication.

The strength of the mitogenomic data set described above is the inclusion of all protein-coding mitochondrial loci at the expense of taxonomic representation. To evaluate the impact of taxonomic sampling in this dataset, we assembled a second mitochondrial DNA dataset by adding the two species of beavers to the taxon-rich matrix reported by Hafner *et al.* (2007). The sequences of the 12s and 16s ribosomal loci and of COX1 for beavers were obtained from the aforementioned mitogenomes. This expanded dataset included representatives from all genera and 43 species, with COX1 missing for *Cratogeomys estor* and 12s missing for *Heterogeomys dariensis*.

Ultraconserved elements (UCEs). We used the UCEs reported by Swanson *et al.* (2019) for *Castor canadensis*, *Heteromys oasicus*, *Dipodomys ordii*, and *Cratogeomys planiceps* (BioSample/genome accessions at NCBI: SAMN10715119, SAMN10715105, GCA_000151885.2_Dord_2.0, and SAMN10715121, respectively). In addition, we used the software phyluce v1.7.3 (Faircloth 2016) to mine the reference genomes of *Thomomys bottae* (Museum of Vertebrate Zoology: voucher MVZ:Mamm:240275; reference genome: mThoBot1.0.p; Genbank accession: GCA_024803745.1; Voss *et al.* 2024), *Dipodomys ordii* (Dord_2.0, GCF_000151885.1); *Dipodomys merriami* (MVZ:Mamm:240054, mDipMer1.0.p, GCA_024711535.1), *Dipodomys spectabilis* (Auburn University:Male_0828, ASM1905484v1, GCA_019054845.1); Harder *et al*. 2022), *Dipodomys stephensi* (Broad Institute:BS19, DipSte_v1_BIUU, GCA_004024685.1), and *Perognathus longimembris* (San Diego Zoo Wildlife Alliance:PPM17, ASM2315922v1, GCA_023159225.1; Kozak *et al.* 2024).

The number of UCE loci recovered per taxon were as follows: *Castor canadensis* (3,700), *Dipodomys merriami* (3,643), *D. ordii* (3,947), *D. spectabilis* (3,953), *D. stephensi* (3,966), *Heteromys oasicus* (2,359), *Perognathus longimembris* (3,856), *Cratogeomys planiceps* (2,852), and *Thomomys bottae* (3,837). The final, full UCE dataset, allowing for up to two missing taxa per locus, included 3,991 loci.

Analyses were also carried out separately on a reduced UCE dataset of the 1,750 loci that were available for all taxa.

Alignments and phylogenetic analyses. Alignments of each of the 4 datasets were done using MAFFT v7.520 (Katoh and Standley 2013).

Phylogenetic analysis for concatenated data was carried out in IQ-TREE 2.2.5 (Minh *et al.* 2020), separately for mitogenomes, the three mitochondrial loci used by Hafner *et al.* (2007), and the full (3,991 loci) or reduced (1,750 loci) UCE data. For each data set, each locus was treated as a separate partition, for which model selection was carried out independently with ModelFinder within IQ-TREE (Kalyaanamoorthy *et al.* 2017). ModelFinder uses a greedy strategy that first considers a full partition model and sequentially merges partitions, so long as there is no significant loss in model fit.

To circumvent high consumption of RAM memory, node support with the UCEs dataset was assessed considering a single partition (with TVM+F+I+R5, a transversion model with unequal base frequencies estimated empirically, a fraction of invariant sites, and five categories of rates to allow for rate heterogeneity among sites– selected as the best model with ModelFinder, using the Bayesian Information Criterion).

Node support for each of the four datasets was carried out with 1,000 pseudo-replicas with an ultrafast bootstrap approximation with Maximum Likelihood (ML) in IQ-TREE; the best ML tree was annotated with percent bootstrap support for each node.

Independently for each UCE locus, model selection was carried out with ModelFinder as described above, and a Maximum Likelihood (ML) gene tree was recovered. Using those trees as input, the species tree was obtained using a multispecies coalescent-based method implemented in wASTRAL-unweighted v1.16.3.4 (Zhang *et al.* 2018; Zhang and Mirarab 2022). Local posterior probability (local PP) which is the probability that a branch is true given the set of gene trees (Sayyari and Mirarab 2016) was used as an estimate of support for each node.

Results

Mitochondrial phylogenies. The dataset for the 13 protein-coding loci for 13 taxa consisted of 11,491 sites, of which 5,061 were parsimony-informative and 1,009 were singletons. The ML phylogeny recovered from this dataset is presented in Figure 2. Within the superfamily Geomyoidea, the bootstrap consensus tree shows Heteromyidae and Geomyidae as sister taxa, *i. e.*, the two families are recovered as reciprocally monophyletic. However, support for reciprocal monophyly is very low (55 % bootstrap value). This phylogeny shows support for monophyly of the Geomyidae, as expected from previous phylogenetic efforts. As for internal relationships within Heteromyidae, the monophyly of *Dipodomys* and of the Perognathinae (*Perognathus* + *Chaetodipus*) are well supported.

Our second mitochondrial dataset (that adds the 12s, 16s, and COX1 loci of beavers to the geomyoid matrix produced by Hafner *et al.* 2007, for a total of 43 taxa) included 4,251 sites, of which 1,166 were parsimony-informative and 448 were singletons. The Maximum Likelihood phylogeny based on this data set provides a complementary perspective on the mitochondrial phylogeny of castorimorphs, with strong taxon representation at the expense of additional loci. The resulting tree (Figure 2) shows strong support (100 % bootstrap) for Geomyidae, and the subfamily Heteromyinae. Support for the Dipodomyinae and Perognathinae is moderate (89 % and 93 % bootstrap support, respectively). The Heteromyidae are recovered as paraphyletic relative to Geomyidae. Specifically, the tree includes a clade formed by Perognathinae, Dipodomyinae and Geomyidae to the exclusion of Heteromyinae, but with low bootstrap support (81 %). The sister taxon relationship between Dipodomyinae and Geomyinae is even less supported (bootstrap <50 %).

Additional information for each locus, the partition scheme, and selected substitution models, as well as details of the results, are provided in the Supplementary materials.

Phylogeny based on Ultraconserved elements (UCEs). The 3,991 UCE loci had an average length of 725 sites (range: 368 to 2,290), for a total of 2,895,348 sites, of which 139,516 were parsimony-informative and 350,453 were singletons (additional information of each locus is provided in Supplementary Table 1). The phylogeny based on the full UCE data using a multi-species coalescent method (as implemented in wASTRAL) is presented in Figure 3. Kangaroo rats (four species of *Dipodomys*), pocket mice (*Heteromys oasicus* + *Perognathus longimembris*), and pocket gophers (*Cratogeomys planiceps* + *Thomomys bottae*) are strongly supported as monophyletic units (local Posterior Probability of 1). In addition, pocket gophers are recovered as sister to pocket mice (local PP of 1) to the exclusion of kangaroo rats. A phylogeny based on a concatenation of the UCE loci analyzed by Maximum Likelihood (results not shown) recovers all these clades, including the sister taxon relationship between pocket gophers and pocket mice (to the exclusion of kangaroo rats) with 100 % bootstrap support.

Both the multi-species coalescent (wASTRAL) and concatenated (Maximum Likelihood) analyses recover the pair formed by *D. ordii* and *D. stephensi* with local PP of 1. However, wASTRAL places *D. merriami* as sister to this pair, but support for this relationship is low (local PP = 0.67);

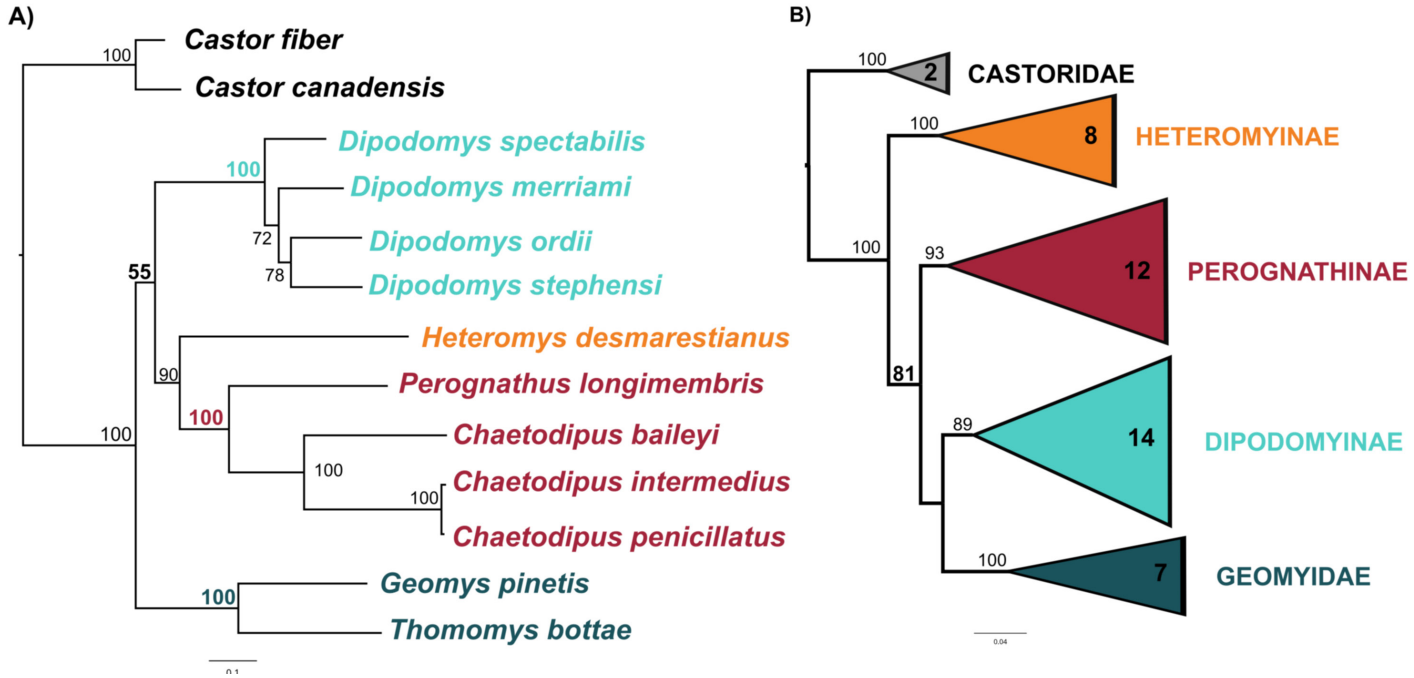

Figure 2. Phylogenies of castorimorph rodents based on mitochondrial DNA sequences; bootstrap values for nodes shown if > 50 %. A) Maximum Likelihood phylogeny based on the 13 protein-coding loci encoded by the mitochondrial genome. B) Reanalysis of Hafner *et al.* (2007) data for 12s, 16s, and COX1, after including the 2 beaver species as outgroups to the Geomyoidea. The number of species included for each family or subfamily is shown within the corresponding triangles.

Table 1. Classification and diversity of extant castorimorphs (suborder Castorimorpha, in Wilson and Reeder (2005); Fabre *et al.* (2015); infraorder Castorimorphi in D'Elía *et al.* (2019). The number of species per genus was obtained from the Mammal Diversity Database (2023), v1.12.1, released 30 Jan 2024.

Superfamily	Family	Subfamily	Tribe	Genus	Number of species	Common name
	Castoridae			*Castor*	2	beavers
Geomyoidea	Heteromyidae	Heteromyinae		*Heteromys*	17	spiny pocket mice
		Perognathinae		*Perognathus*	10	pocket mice
				Chaetodipus	20	pocket mice
		Dipodomyinae		*Microdipodops*	2	kangaroo mice
				Dipodomys	20	kangaroo rats
Geomyoidea	Geomyidae		Geomyini	*Cratogeomys*	7	pocket gophers
				Geomys	13	"
				Heterogeomys	7	"
				Orthogeomys	1	"
				Pappogeomys	1	"
				Zygogeomys	1	"
			Thomomyini	*Thomomys*	12	"

in contrast, Maximum Likelihood recovers *D. spectabilis* as sister to the *D. ordii* + *D. stephensi* pair with strong (local PP = 0.98) support.

The reduced UCE dataset included 1,750 loci with an average length of 729 sites, for a total of 1,276,436 sites, of which 68,826 were parsimony-informative and 143,039 were singletons. Using both wASTRAL and Maximum Likelihood analyses, this dataset produced topologies that were identical and with local PP of 1 and 100 bootstrap support, respectively, for the monophyly of kangaroo rats, pocket mice, and pocket gophers; the sister-taxon relationships of pocket mice and pocket gophers to the exclusion of kangaroo rats was also well supported in both cases (local PP = 1; 100 % bootstrap).

As for the relationships among kangaroo rats obtained from the reduced UCE dataset, each of the methods recovered the same topology obtained with the full dataset. However, whereas support for the topology obtained with wASTRAL increased to a local PP of 0.76, support for the Maximum Likelihood phylogeny dropped from 98 % to 64 %.

Additional information of each locus, the partition scheme, and selected substitution models, as well as details of the results, are provided in the Supplementary materials.

Discussion

Phylogenetics of Geomyoidea: where do we stand? The monophyly of the family Geomyidae is strongly supported by morphological studies (*e. g.*, Wahlert 1985) and, unsurprisingly, by virtually all molecular phylogenies (see Introduction; Figure 1), including our reanalysis of the data from Hafner *et al.* (2007), the tree obtained from mitogenomic data (Figure 2), as well as our phylogenies based on UCEs (Figure 3), with the caveat that the latter have only one Geomyini and one Thomomyini. The reciprocal monophyly of these two tribes is also fully supported by molecular phylogenies (*e. g.*, Spradling *et al.* 2004; Upham *et al.* 2019).

Previous molecular phylogenetic studies have shown increasing support for the subfamilies of Heteromyidae (Figure 1). The Dipodomyinae (*Dipodomys* + *Microdipodops*) are well supported in the studies of Fabre *et al.* (2012) and Upham *et al.* (2019), and the Heteromyinae (*Heteromys*, including *Liomys* as a junior synonym) are well supported in Hafner *et al.* (2007) and subsequent studies. Although Fabre *et al.* (2012) did not recover a monophyletic Perognathinae (*Perognathus* + *Chaetodipus*), support for the monophyly of this subfamily is strong in both the mitochondrial study of Hafner *et al.* (2007) and the analysis that combines nuclear and mitochondrial loci of Upham *et al.* (2019). Our mitochondrial genomic data (Figure 2) provide strong support for the monophyly of the Perognathinae, with the caveat that taxonomic representation is limited.

In sum, there is good support for the monophyly of each of the four major groups of geomyoids, namely the family Geomyidae and the subfamilies Heteromyinae, Perognathinae, and Dipodomyinae. The relationships among them, however, remain problematic. As discussed above, reciprocal monophyly of Geomyidae and Heteromyidae is recovered in some analyses, including our mitogenomic tree (Figure 2); however, support for that topology is very low (55 % bootstrap). Collapsing the key node results in a trichotomy, with branches leading to Dipodomyinae, Perognathinae + Heteromyinae, and Geomyidae. As such, neither our reanalysis of the data from Hafner *et al.* (2007) with beavers as an outgroup nor our mitogenomic analyses support the reciprocal monophyly of the two families.

As discussed in the Introduction, the alternative hypothesis (paraphyly of Heteromyidae relative to Geomyidae) has been recovered in some, but not all molecular phylogenies, generally with low support (see Figure 1). Our UCE data provide strong support for the sister taxon relationship between pocket mice (Heteromyinae + Perognathinae) and pocket gophers (Geomyidae), to the exclusion of Dipodo-

myinae (Figure 3), with the caveat that taxonomic sampling is limited to eight geomyoids and one beaver as an outgroup. In particular, the genera *Chaetodipus* and *Microdipodops* are not represented in the data. Also missing are several genera of Geomyini, although there is little question about the monophyly of Geomyidae. Interestingly, a topology suggesting that pocket mice (Heteromyinae + Perognathinae) are sister to Geomyidae is recovered in both maximum likelihood analyses of concatenated UCE data and in the coalescent-based phylogenies based on UCE loci. Furthermore, the topology is similar to the one obtained, with less support, by Upham *et al.* (2019), a study that is much stronger in taxonomic representation (see Table 2). It should be noted, however, that, although Upham *et al.* (2019) used four mitochondrial and 24 nuclear loci of Castorimorpha, the representation of most of the nuclear loci is limited to a few species.

Taken collectively, our and earlier studies seem to indicate that mitochondrial sequences cannot resolve relationships among the subfamilies of Heteromyidae and the family Geomyidae. Nuclear data, alone or combined with mitochondrial data, have much greater promise, as shown by Upham *et al.* (2019) and by our analysis of UCE data, both of which lean in support of heteromyid paraphyly, with pocket mice sister to pocket gophers. These results are provisional and will have to be re-examined with a good combination of taxonomic representation and large numbers of loci. We discuss some options to achieve that goal in the next section.

Molecular phylogenetics of Geomyoidea in the genomic era. In principle, PCR amplification and sequencing of the loci used by Upham *et al.* (2019) can provide a more thorough representation of genes across taxa, as required to resolve higher-level relationships within the Geomyoidea. Targeted enrichment protocols, combined with next generation sequencing, provide several alternatives to locus-by-locus sequencing. For example, Bangs and Steppan (2022) developed a set of probes to obtain > 400 loci specifically selected for rodent phylogenetics; importantly, these loci have been shown to be useful at different levels of divergence within rodents. Radseq or ddRADseq is yet another effective method for sampling a few thousand genes (Peterson *et al.* 2012), but the approach appears to work best to examine variation within and between closely related species (*e. g.*, Tomasco *et al.* 2024). Exome capture can potentially provide sequences of the coding regions of thousands of loci, and the approach is adaptable to the needs of each project, especially at the population level (Bi *et al.* 2013). For higher-level relationships, UCE tetrapod probes can effectively capture up to 5,000 loci (McCormack *et al.* 2012; Esselstyn *et al.* 2017; McLean *et al.* 2019; Swanson *et al.* 2019).

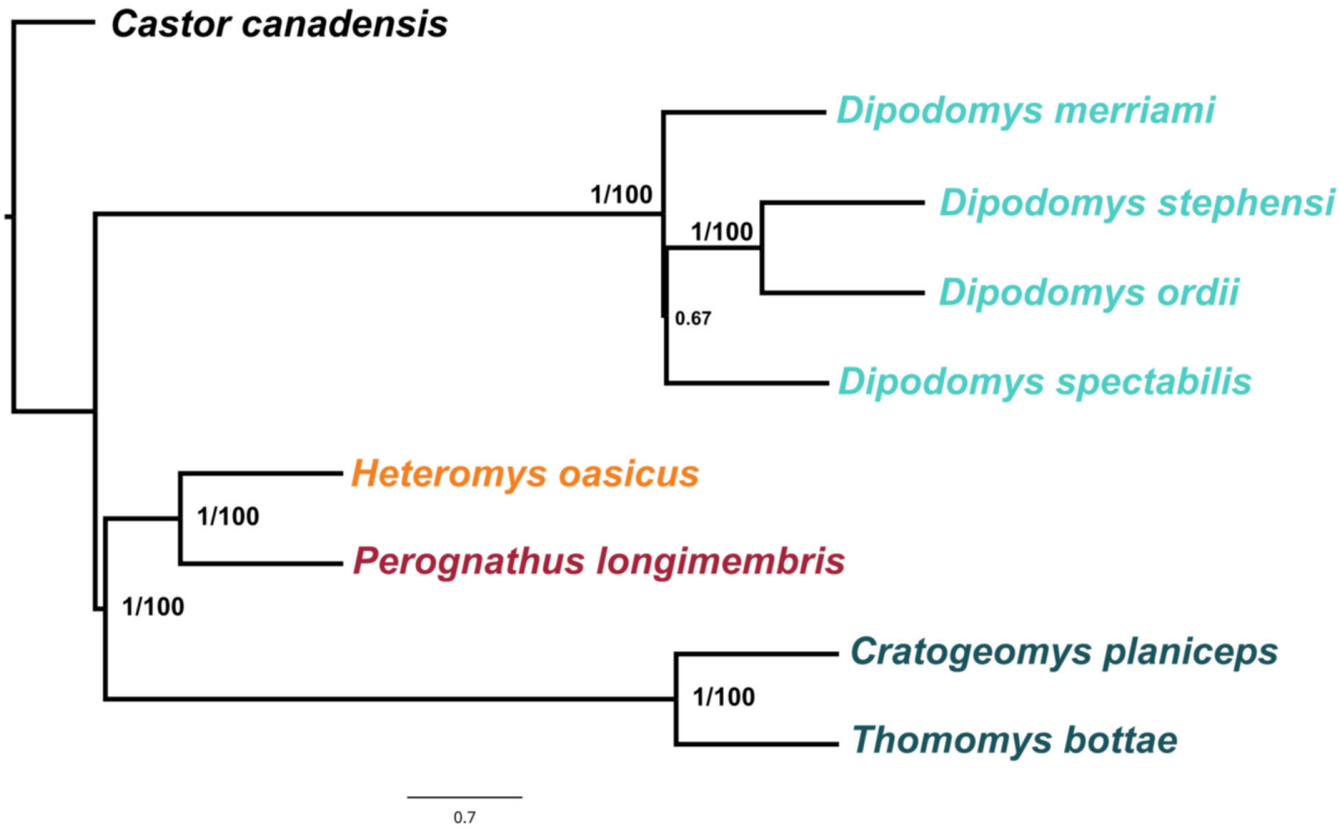

Figure 3. Phylogeny of castorimorph rodents using ultraconserved elements, obtained with wASTRAL and the 3,991 loci dataset. Support values are shown for the local posterior probability (local PP) from wASTRAL and analysis of 1,000 ultrafast bootstrap Maximum Likelihood pseudo-replicates (%). Only local PP support is shown for a node within *Dipodomys*, as this relationship is not recovered with Maximum Likelihood (see Results).

Table 2. Taxonomic and genetic coverage of selected studies of phylogenetic relationships of castorimorphs based on DNA sequences. The number of species per genus (in parenthesis) is taken from the Mammal Diversity Database (2023) in the first column, and from each of the studies in the remaining columns.

Family and genus	Spradling *et al.* 2004	Hafner *et al.* 2007	Montgelard *et al.* 2008	Blanga-Kanfi *et al.* 2009	Fabre et al. 2012	Fabre *et al.* 2015	Upham *et al.* 2019
Castoridae							
Castor (2)			1	1	2	1	2
Geomyidae							
Cratogeomys (7)	5	1	1		8	1	7
Geomys (12)	2		1		8	1	10
Heterogeomys (17)	3				4		
Orthogeomys (1)	1	1			1		11
Pappogeomys (1)	1	1			2		2
Zygogeomys (1)	1	1			1		1
Thomomys (12)	6		1	1	7	1	12
Heteromyidae							
Dipodomys (20)		12	1	1	19	1	21
Microdipodops (2)		2			2		2
Heteromys[a] (17)		8	1		9	1	15
Chaetodipus (20)	1	7			15		17
Perognathus (10)		4			9		9
Total (112)	**20**	**37**	**6**	**3**	**87**	**6**	**109**
Number of loci							
mitochondrial	2	3	2		6		4
nuclear	2		6	6	5	10	24
Total	**4**	**3**	**8**	**6**	**11**	**10**	**28**

a. Includes *Liomys* as a junior synonym.

Any of the loci recovered by these methods can be mined from the growing number of available genomes to supplement sequencing efforts. In the case of UCEs, software is available to extract them from genomes (Faircloth 2016), and the resulting data can be combined with those from UCE studies, as illustrated by our own UCE dataset (see also Swanson *et al.* 2019; Parada *et al.* 2021). The number of available genomes will continue to increase and taxonomic representation will improve along the way. Even full genomes, however, cannot resolve all relationships with limited taxonomic density. As an example, relationships between the three major clades of rodents were not clearly resolved in a recent analysis based on high quality genomes (Foley *et al.* 2023).

To sum up, we think that targeted enrichment and sequencing of nuclear loci in general, and UCEs in particular, provide opportunities to resolve higher level relationships among geomyoids. In general, phylogenetic methods based on multispecies coalescent models appear particularly appropriate to analyze difficult nodes (*e. g.*, Kubatko and Degnan 2007; Liu and Edwards 2009), but challenges persist in the realm of multispecies coalescent methods (*e. g.* (Philippe *et al.* 2011; Meiklejohn *et al.* 2016). Recently, UCEs have been used to explore rapid radiations where short branches between speciation events are documented by few substitutions and may be obscured by extensive incomplete lineage sorting (Esselstyn *et al.* 2017; McLean *et al.* 2019; Parada *et al.* 2021).

Integration of molecular, morphological and paleontological data. It is perhaps not surprising that mitochondrial DNA data or taxonomically sparse nuclear data fail to resolve deep relationships with the Geomyoidea. Hafner *et al.* (2007: Figure 3) estimated that the divergence between Dipodomyinae, Perognathinae and Heteromyinae occurred in the Early Miocene and involved branching events some 20 to 22 MYA, and recent analyses of the fossil record of Geomyoidea are consistent with an Early Miocene, or even Late Oligocene divergence of the group (Samuels *et al.* 2023, and references therein). Short intervals between deep divergence events are typically difficult to recover with limited genetic sampling.

As summarized in the Introduction, proposals of heteromyid paraphyly relative to geomyids are not new but encompass three inter-related issues. First, paraphyly may result from the inclusion of stem fossil taxa in the Heteromyidae. For example, Korth (1994:182) noted that, "If *Proheteromys* is considered ancestral to the geomyids and is within the Heteromyidae, this also implies the derivation of the Geomyidae from a heteromyid ancestor."

Second, and restricting the focus on living geomyoids, crown Heteromyidae have at times been recovered as paraphyletic relative to Geomyidae. Early proposals of heteromyid paraphyly were based on qualitative assessments of dental traits (Wood 1931; Lindsay 1972). In general, as other characters were included, subsequent morphological analyses have supported reciprocal monophyly of crown het-

eromyids and geomyids (Wahlert 1985; Ryan 1989; Flynn *et al.* 2008), with the notable exception of an analysis of stapedial arteries (Brylski 1990).

Third, given the historical uncertainty in the placement of Geomyoidea among rodents, character polarity has been difficult to establish. Even recent phylogenetic reconstructions centered on fossil geomyoids (Samuels *et al.* 2023) do not include beavers as an outgroup. The sister-taxon relationship of Geomyoidea and Castoridae, which has been known for about two decades, has not been put to use to this end but is key to understanding the higher-level relationships of the Geomyoidea. It appears that the time is ripe to integrate the wealth of morphological information about living and fossil Geomyoidea with molecular phylogenetic data.

Ultimately, we want to infer phylogenetic relationships to help us understand how evolution has produced emblematic critters of North America, such as pocket mice, pocket gophers and kangaroo rats and mice, with their wealth of morphological, physiological, behavioral and ecological adaptations, building upon the remarkable insights provided by Jim Patton in his studies of these fascinating organisms.

Acknowledgments

Our understanding of the evolutionary biology of the Geomyoidea has been greatly impacted by Jim Patton's work throughout his remarkable career, beginning with the articles stemming from his graduate work (Patton 1967), and including the recent, high-quality genomes of *Perognathus longimembris* (Kozak *et al.* 2024) and *Thomomys bottae* (Voss *et al.* 2024), from which we extracted some of the data used in this study. We wish to express our admiration of Jim Patton for the breadth and impact of his scholarly contributions in mammalogy and evolutionary biology, for his broader contributions to the development of science internationally, and for his generosity as a mentor of innumerable students and scholars. In addition, Enrique Lessa wishes to express his personal gratitude to both Jim and Carol Patton for their encouragement and multi-faceted support throughout his career. We are grateful to Marjorie Matocq and an anonymous reviewer for their suggestions on earlier versions of this article.

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Supplementary materials

The datasets (two mitochondrial, 2 UCE), partition schemes, and additional details on the various loci, along with the results of phylogenetic analyses are available at https://doi.org/10.6084/m9.figshare.28046777.v1.

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Genus-level review of pocket gophers in the family Geomyidae

Sergio Ticul Álvarez-Castañeda[1]*, and Cintya A. Segura-Trujillo[2]

[1] Centro de Investigaciones Biológicas del Noroeste, S.C. Av. Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, CP. 23096, La Paz. Baja California Sur, México. Email: sticul@cibnor.mx (STA-C).

[2] Departamento de Bienestar y Desarrollo Sustentable, Centro Universitario del Norte, Universidad de Guadalajara. Carretera Federal No. 23, Km. 191, CP. 46200, Colotlán. Jalisco, México. Email: cintya.segura@academicos.udg.mx (CAS-T).

* Corresponding author: https://orcid.org/0000-0002-2689-8758.

Pocket gophers (Geomyidae) comprise a well-studied family at the species level but need an updated revision at the generic level because studies of each genus have applied different data sets and different criteria for recognizing distinct taxa. Pocket gophers thrive from temperate Canada south to Panama and Colombia, where they inhabit various habitats, including temperate forests, prairies, steppes, hot and cold deserts, and subtropical and tropical areas. The taxonomy at the genus and species levels underwent many changes in the early twenty-first century due to use of different sequencing methodologies. This article builds upon those analyses to review genus-level relationships within the Geomydae. Specifically, we analyzed the sequences available in Genbank for members of the family Geomyidae (Cytb for 47 species and COI for 33 species). We conducted different phylogenetic analyses; in all cases, genera were classified into monophyletic groups associated with the tribes Geomyini and Thomomyini. In the Thomomyini, the genus *Thomomys* was recognized with two genera, *Megascapheus* and *Thomomys*, which are more genetically distinct than many other genera. In the Geomyini, each genus and subgenus are distinct monophyletic groups with very strong support and large *p*-distances. The Mississippi River appears to function as an important geographic barrier within *Geomys*, with marked genetic differentiation between populations on the eastern and western sides of the river. Collectively, our analyses based on mtDNA sequences suggest that a more detailed revision employing multiple data sets is needed for the genera within the Geomyidae.

Las tuzas (Geomyidae) comprenden una familia bien estudiada a nivel de especies, pero necesitan una revisión actualizada a nivel de género, debido a que los estudios de cada género han utilizado diferentes conjuntos de datos y criterios para reconocer los distintos taxa. Las tuzas se distribuyen desde Canadá hasta Panamá y Colombia, en diversos hábitats, incluyendo bosques templados, praderas, estepas, desiertos fríos y calientes, así como áreas subtropicales y tropicales. La taxonomía a nivel de género y especie tuvó muchos cambios a principios del siglo veinte y uno debido al uso de diferentes metodologías de secuenciación. Este artículo se basa en esos análisis para revisar las relaciones a nivel de género dentro de los Geomyidae. Específicamente, analizamos las secuencias disponibles en Genbank para especies de la familia Geomyidae (Cytb para 47 especies y COI para 33 especies). Realizamos diferentes análisis filogenéticos; en todos los casos, los géneros fueron clasificados en grupos monofiléticos asociados con las tribus Geomyini y Thomomyini. En los Thomomyini, el género *Thomomys* fue reconocido con dos géneros, *Megascapheus* y *Thomomys*, que son genéticamente más distintos que muchos otros géneros. En los Geomyini, cada género y subgénero son grupos monofiléticos distintos con un fuerte apoyo y grandes distancias p. El río Misisipi parece funcionar como una importante barrera geográfica dentro de *Geomys*, con una diferenciación genética notable entre las poblaciones en los lados este y oeste del río. Colectivamente, nuestros análisis basados en secuencias de mtDNA sugieren que se necesita una revisión más detallada utilizando múltiples conjuntos de datos para los géneros dentro de los Geomyidae.

Keywords: *Geomys*; *Heterogeomys*; *Megascapheus*; genus; taxonomy; *Thomomys*.

Introduction

The family Geomyidae is endemic to the Americas, being distributed from temperate Canada south to Panama and Colombia. Collectively, geomyids occupy a wide range of habitats, from temperate forests, prairies, steppes, hot and cold deserts, and subtropical and tropical areas (Hafner 2017). Over most of their distributions, the different genera of Geomyidae have allopatric distributions (Hall 1981; Hafner 2017). The only areas where two genera are sympatric are in the highlands of central and northern México, where different species of *Thomomys* and *Cratogeomys* or *Cratogeomys* and *Zygogeomys* co-occur (Russell 1968a; Hall 1981; Patton 2005). The genus- and species-level taxonomy of the Geomyidae underwent multiple changes in the early twenty-first century based on analyses of mitochondrial DNA (mtDNA) and karyotype differences. For example, *Cratogeomys* and *Pappogeomys*, both previously considered subgenera of *Cratogeomys*, were elevated to generic status. Similarly, *Heterogeomys* and *Orthogeomys*, both previously considered subgenera of *Orthogeomys,* were elevated to generic status (Russell 1968a; Hall 1981; Demastes *et al.* 2002; Spradling *et al.* 2016).

While the taxonomy and systematics of the Geomyidae have been studied by multiple authors over the past few decades (*e. g.*, Elliot 1903; Russell 1968a; Honeycutt and Williams 1982; DeWalt *et al.* 1993; Patton 2005; Álvarez-Castañeda 2010; Spradling *et al.* 2016), many of these studies have focused on a specific genus or group of species within this family. For this reason, there are no consistent criteria that can be used to resolve taxonomic issues for all mem-

bers of this family. Pocket gophers are a relatively well-studied group at the species level, but a comprehensive revision is needed at the genus level and this may require reconsideration of the criteria used to distinguish between different genera.

Currently, the family Geomyidae is represented by two tribes (Russell 1968a). The tribe Thomomyini includes only the genus *Thomomys*, with two recognized subgenera, *Megascapheus* and *Thomomys* (Elliot 1903). In contrast, the tribe Geomyini (Russell 1968a) includes six genera – *Cratogeomys, Geomys, Heterogeomys, Orthogeomys, Pappogeomys,* and *Zygogeomys*. Of these, only *Heterogeomys* contains subgenera, namely *Heterogeomys* and *Macrogeomys* (Russell 1968a). Multiple taxonomic assessments have been completed for each of the currently recognized genera, as exemplified by the following: *Cratogeomys* (Russell 1968b; DeWalt *et al.* 1993; Hafner *et al.* 2004, 2005, 2008), *Geomys* (Merriam 1895; Hall and Kelson 1959; Russell 1968a; Tucker and Schmidly 1981; Heaney and Timm 1983; Baker *et al.* 1989; Block and Zimmerman 1991; Jolley *et al.* 2000; Sudman *et al.* 2006; Chambers *et al.* 2009), *Heterogeomys* (Nelson and Goldman 1929; Russell 1968a; Hall 1981; Patton 2005; Spradling *et al.* 2016), *Orthogeomys* (Nelson and Goldman 1929; Russell 1968a; Hall 1981; Patton 2005; Spradling *et al.* 2016), *Pappogeomys* (Nelson and Goldman 1934; Russell 1968b; Honeycutt and Williams 1982; Demastes *et al.* 2002), *Thomomys* (Hall and Kelson 1959; Anderson 1966, 1972; Patton and Dingmen 1968; Russell 1968a; Thaeler 1968a, b, 1972, 1977, 1980; Hoffmeister 1969, 1986; Patton 1973, 1993, 2005; Thaeler and Hinesley 1979; Patton and Smith 1981, 1990; Hall 1981; Patton *et al.* 1984; Álvarez-Castañeda 2010; Hafner *et al.* 2011; Trujano-Álvarez and Álvarez-Castañeda 2013; Mathis *et al.* 2013a, 2013b, 2014; Álvarez-Castañeda *et al.* 2017; Bradley *et al.* 2023), and *Zygogeomys* (Merriam 1895; Russell 1968a; Hall 1981).

The systematics of the Geomyidae were first established during the late nineteenth to mid- twentieth centuries based on morphology (Merriam 1895; Russell 1968a, b), before the advent of DNA sequencing technologies. Indeed, many of the studies that have contributed to the current taxonomy of the family (see above) pre-date the use of genetic information. Subsequent revisions within each genus or species complex that have employed genetic data have tended to be conducted by multiple groups of researchers employing different criteria to identify genetically distinct taxonomic units. As a result, a comprehensive review of the family that applies consistent sequenced-based criteria to distinguish taxonomic units is lacking. Although mtDNA, nDNA, and karyotypes have all been used to explore geomyid taxonomy (for example, Hafner *et al.* 2004, 2005, 2008, 2009; Spradling *et al.* 2016; Sudman *et al.* 2006; Chambers *et al.* 2009), the most widely employed genetic marker is the mitochondrial cytochrome b (Cytb) locus. Accordingly, the primary objective of this study was to use Cytb data to evaluate genus-level taxonomic and systematic relationships with the Geomyidae. Delineating generic boundaries requires a well-resolved phylogeny that includes as many species as possible to *i*) generate a comprehensive overview of current generic names and their type species and *ii*) clarify generic boundaries and the species that they contain. This article uses Cytb data to create the taxonomic and systematic background required for a rigorous revision generic-level classifications of pocket gophers.

Materials and methods

Sampling and sequencing. Previous studies have generated cytochrome b (Cytb) sequences for nearly all species of geomyids ($n = 47$ species) as well as cytochrome oxidase subunit 1 (COI) sequences for a somewhat smaller subset of species ($n = 33$ species); all of these sequences are available in GenBank (Supplementary Material 1). All sequences available for geomids were aligned using the MEGA 11 software package (Tamura *et al.* 2021). Sequences containing intermediate stop codons were discarded. Analyses were performed using the sequences obtained from both genes. To optimize computational time, we analyzed a subset of five specimens from each monophyletic group, which was average number of samples per species the (Supplementary Material 2); preference was given to sequences that have been used in published taxonomic revisions. Fewer than five sequences were available for some species (Supplemetary Material 2); we were unable to locate sequences for *Thomomys idahoensis* and *T. clusius*.

Phylogenetic analyses. Analyses were conducted based on a 1,141-bp fragment of Cytb ($n = 169$ sequences) and a 1,544-bp fragment of COI ($n = 88$ sequences; Supplementary Material 1). Because sequence from both Cytb and COI were not available for all species, data from each locus were analyzed separately. Our first analysis assessed the monophyly of each species based on up to five sequences per species. Since sympatry has not been reported for species in the same genus, the source of each sequence was reviewed in detail to avoid confusion between species or potential misidentifications. When multiple sequences were available, preference was given to sequences from localities located farthest from the distribution limits of other species in the same genus; in all cases, efforts were made to select sequences that clearly represented the known geographic distribution of the species in question. Some species are represented by outdated names in GenBank; in some cases, we changed the name of the species following Álvarez-Castañeda (2024) and Bradley *et al.* (2023; see Supplementary Material 1). Once the monophyly of each species was demonstrated, one sequence per species was selected to construct a representative tree for the family.

Sequence alignments were performed using the MUSCLE software package with default parameters (Edgar 2004). The most suitable evolutionary model for our data set was identified dusing the model comparison software MrModeltest ver. 2 (Nylander 2004) under the Akaike Information Criterion (AIC). Phylogenetic relationships

were assessed for each locus using neighbor-joining (NJ), unweighted pair group method with arithmetic mean (UPGMA), maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI) optimality criteria. Phylogenetic reconstructions were conducted in PhyML (Guindon *et al.* 2010), MEGA version 11 (Tamura *et al.* 2021), and PAUP* version 4.0b (Swofford and Sullivan 2003). A bootstrap consensus tree was inferred from replicates based on uniform rates of the General Time Reversible (GTR) substitution model. Values for percent sequence divergence within and between species were estimated using the uncorrected *p*-distance parameter model in PAUP. Nodal support was assessed with bootstrap analyses, including a fast heuristic procedure with 1,000 pseudo-replicates (Felsenstein 1985). A Bayesian inference analysis coupled with Markov Chain Monte Carlo (BMCMC) inference was performed in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). We carried out two independent BMCMC analyses, each consisting of four chains. Each Markov chain was started from a random tree and run for 10 million generations using the default flat priors, sampling trees every 1,000 generations. Sequence evolution model parameters were treated as unknown variables with uniform default priors and were estimated as part of the analysis. The first 40 % of generations were conservatively deleted as burn-in. Sequences for *Chaetodipus californicus*, *Dipodomys agilis*, *Heteromys nelsoni*, *Liomys pictus*, *Microdipodops pallidus*, and *Perognathus flavescens* were used as outgroups. The outgroup specimens were selected partly following the study of Alexander and Riddle (2005); Genbank accession numbers for outgroup sequences are provided in the supplementary material.

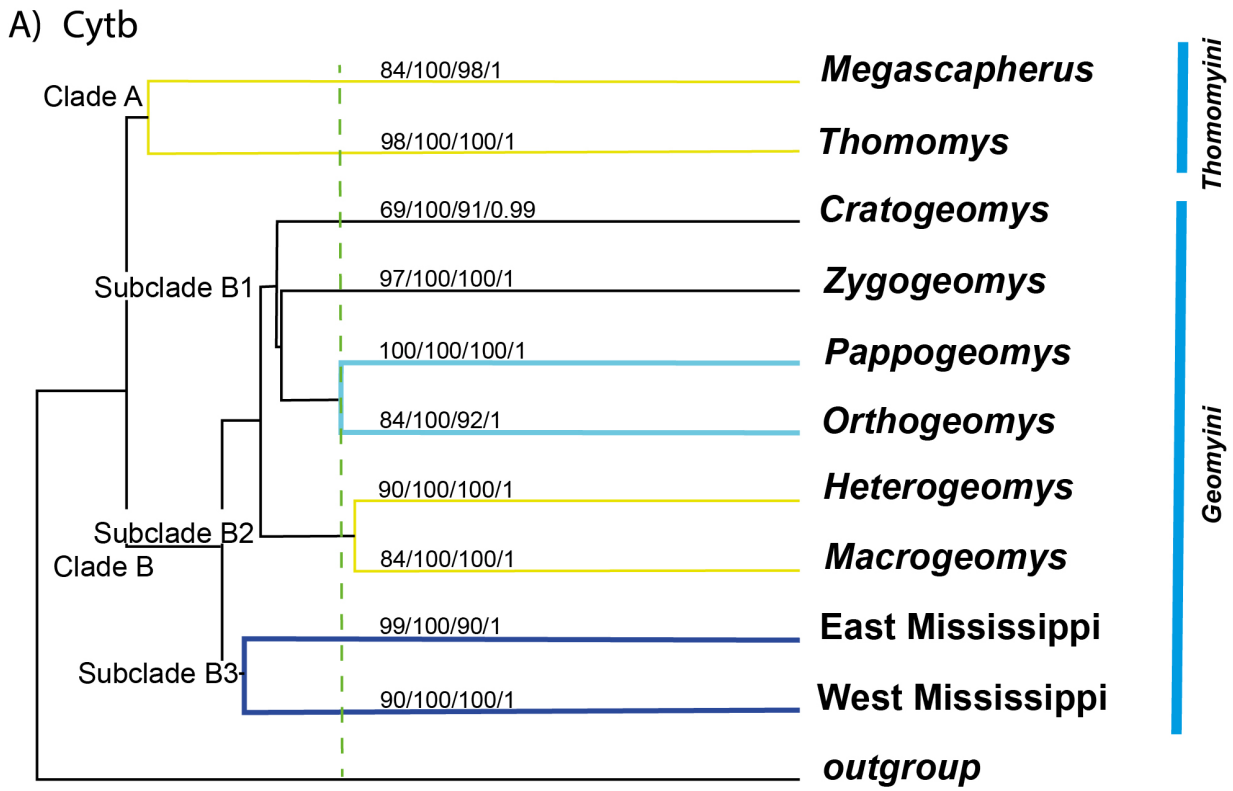
Time calibration. Divergence times between taxa were estimated using BEAST2 v2.6.7 (Bouckaert *et al.* 2019). For each locus, we implemented three separate Markov Chain Monte Carlo (MCMC) chains to generate a gene tree, with each chain running for 10 million generations. Samples were collected every 1,000 generations to assess the posterior distribution. We set specific priors, including the processed Yule speciation model to account for branching rates, a strict molecular clock to enforce constant rates of evolution across lineages, and a random starting tree to avoid biasing the results (Gernhard *et al.* 2008). No deep data for the family Geomyidae were found as calibration points. However, because Heteromyidae is the sibling family and a Bayesian divergence dating analysis exists using combined 3-gene data (12S, 16S, and COI), we used 15.9–12.5 mya for Dipodomyinae, 22–20 mya for Perognathinae and 15.2 mya for Heteromyinae (Hafner *et al.* 2007). To achieve phylogenetic analyses as similar as possible to those reported by Hafner *et al.* (2007), we used the same specimens in our analyses. After running the chains, we used TreeAnnotator version 10.5.0 to summarize the results and construct a consensus tree. Notably, we applied a burn-in period of 1,000 states to remove any initial inconsistencies in chain convergence and to focus on the most stable estimates of the phylogenetic relationships.

Results

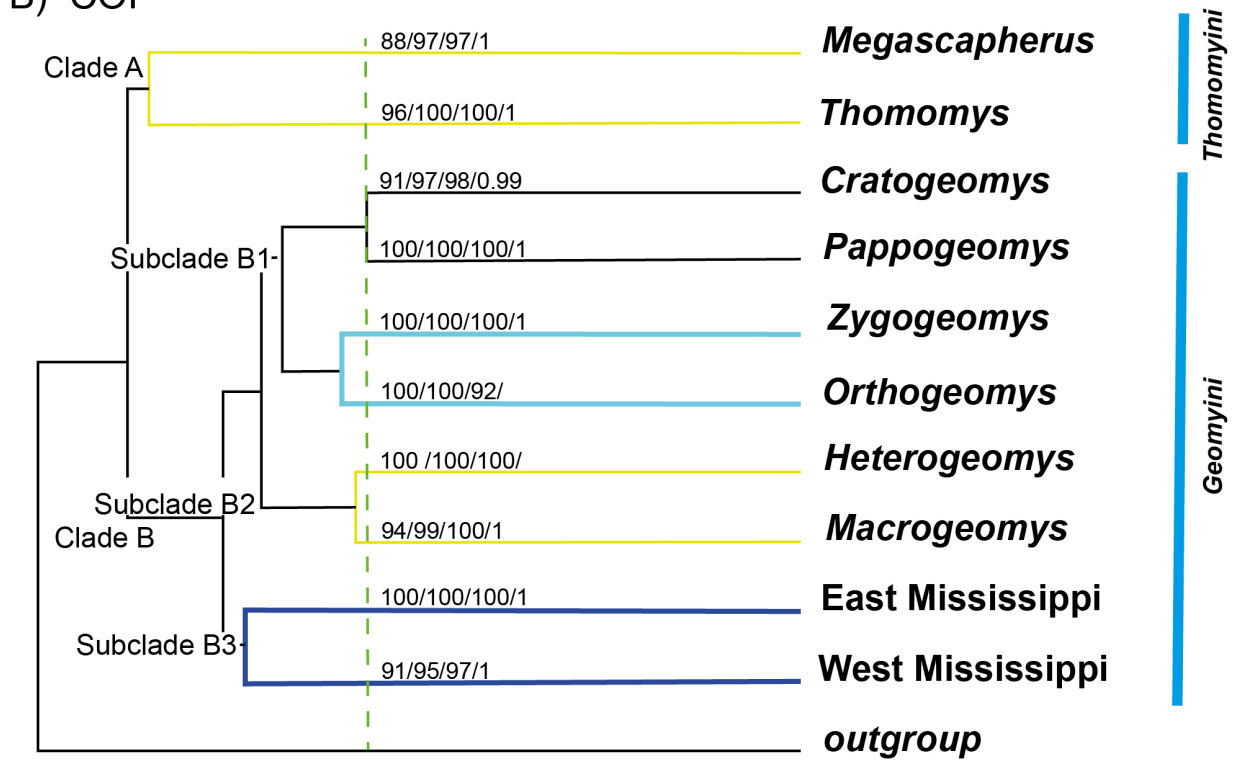
Phylogenetic analyses. The most appropriate evolutionary model for our phylogeny reconstruction was the GTR+I+G model. The model parameters were I = 0.3872 and G = 0.7090, lnL = 28961.9492, k = 10, AIC = 57943.8984. The base frequencies were *A* = 0.3689, *C* = 0.3012, *G* = 0.0502, *T* = 0.2796, and the relative substitution rates were *A–C* = 0.4558, *A–G* = 9.6062, *A–T* = 0.4878, *C–G* = 0.4080, *C–T* = 5.7622, and *G–T* = 1.0000. All phylogenetic reconstructions for Cytb and COI indicated that Geomyidae is a monophyletic family containing ten monophyletic subgroups, each of which is markedly divergent from the others and is strongly supported by bootstrap values (Figure 1; Supplementary Material 3). The neighbor-joining (NJ), unweighted pair group method with arithmetic mean (UPGMA), maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI; Supplementary Material 3) analyses all produced trees with similar topologies. The same primarly clades were recovered for both the Cytb and COI analyses (Figure 1).

Analyses of both loci revealed that all genera were sorted into two monophyletic groups, each associated with a recognized tribe within the family, namely the Thomomyini (Clade A) and the Geomyini (Clade B), as proposed by Russell (1968a). The tribe Thomomyini includes the single genus *Thomomys*, which consists of two previously recognized subgenera: *Megascapheus* and *Thomomys*. Each subgenus, in turn, contains multiple reciprocally monophyletic groups that are differentiated at the same level and characterized by a high degree of dissimilarity (Tables 1 and 2). Many groups have distinctive morphological characteristics that can be used to differentiate them (see appendix 1). The tribe Geomyini (Clade B) includes three subclades. Subclade B1 consists of four monophyletic groups: *Cratogeomys*, *Pappogeomys, Orthogeomys*, and *Zygogeomys.* This was the only subclade to display differences in tree topology between the Cytb and COI sequences. Subclade B2 is monophyletic and contains the subgenera *Heterogeomys* and *Macrogeomys*. The final subclade (B3), which includes all species of *Geomys*, is split into two monophyletic groups that are distributed on the eastern versus western sides of the Mississippi River (Figure 1).

Percentage of uncorrected p-*distance.* Percent sequence divergences between species for Cytb and COI sequences analyses are shown in Tables 1 and 2, respectively; percent divergences within species are shown in Table 3. The least divergence between species was found in *Cratogeomys*, with p-distances ranging from 2.35 % between *C. tylorhinus* and *C. fumosus* to 5.00% between *C. perotensis* and *C. merriami*. When all ten monophyletic sub-groups of geomyids were considered, percent sequence divergence for Cytb ranged from *p* = 11.09 % to *p* = 21.64 % (Table 1); for COI sequences, these values were *p* = 4.48 % and *p* = 18.62 % (Table 2). In Clade A, the difference between the two subgenera was 19.92 % (Table 1). In Clade B, sub-clade B1 contains four monophyletic units, each consisting of a single



Maximum-parsimony / neighbor-joining bootstrap / maximum-likelihoods bootstrap suport values / Bayesian posterior probabilities



Maximum-parsimony / neighbor-joining bootstrap / maximum-likelihoods bootstrap suport values / Bayesian posterior probabilities

Figure 1. Phylogenetic analysis based on 46 specimens (1,141 bp) using cytochrome b gene sequences. The sequences represent individuals of different species belonging to the family Geomyidae. This tree supports the monophyly of ten clades within the Geomyidae. Each clade represents one genus.

Table 1. Pairwise percentage of genetic differences based on cytochrome b (Cytb) across genera and subgenera in the family Geomyidae.

		1	2	3	4	5	6	7	8	9	10
1	*Cratogeomys*										
2	*Pappogeomys*	15.70%									
		14.04-16.77									
3	*Zygogeomys*	15.99%	15.50%								
		13.74-18.42	14.73-15.95								
4	*Orthogeomys*	15.97%	13.03%	15.94%							
		13.27-18.42	6.76-15.48	15.24-16.23							
5	*Heterogeomys*	16.47%	16.51%	16.45%	14.81%						
	(subgenus)	13.10-18.70	14.86-18.19	15.87-17.11	2.64-17.67						
6	*Macrogeomys*	16.36%	16.54%	15.38%	15.36%	11.09%					
	(subgenus)	14.65-17.72	15.96-17.01	14.88-15.79	14.43-16.32	9.11-11.67					
7	*Geomys*	17.95%	16.34%	16.65%	16.66%	18.23%	17.09%				
	(west Mississippi)	16.23-20.32	15.28-17.37	14.56-18.49	12.65-19.34	16.27-19.74	15.92-18.21				
8	*Geomys*	17.55%	16.56%	16.31%	14.94%	17.62%	16.85%	16.76%			
	(east Mississippi)	16.32-18.74	16.36-16.83	16.19-16.41	12.74-16.80	15.96-18.60	16.23-18.07	15.48-17.83			
9	*Megascapheus*	20.71%	19.85%	20.27%	21.64%	20.59%	18.96%	20.15%	19.72%		
	(subgenus)	18.77-22.98	18.49-21.71	18.74-22.15	18.95-25.40	17.60-22.09	17.47-21.48	17.98-22.65	17.33-21.75		
10	*Thomomys*	20.74%	21.15%	19.61%	21.63%	20.31%	19.18%	20.91%	20.02%	19.92%	
	(subgenus)	19.47-21.99	20.59-21.75	18.78-21.40	20.44-23.89	17.52-22.55	16.97-21.23	19.65-22.46	19.38-20.53	18.16-21.23	
0	outgroup	23.31%	22.92%	22.80%	23.82%	22.61%	21.66%	23.15%	23.01%	23.36%	24.01%
		20.88-25.50	21.23-25.73	20.58-24.73	21.30-27.79	19.35-24.50	19.48-24.06	11.34-25.81	11.34-25.81	21.63-25.81	22.72-25.36

recognized genus. Percent sequence divergences within these genera are as follows: *Cratogeomys* (p = 15.70 % Cytb, p = 13.09 % COI), *Pappogeomys* (p = 13.03 % Cytb, p = 13.99 % COI), *Orthogeomys* (p = 13.03 % Cytb, p = 14.46 % COI), and *Zygogeomys* (p = 15.38 % Cytb, p = 14.46 % COI). Subclades B2 and B3 contain only two groups each, with p = 11.09 % Cytb and p = 10.44 % COI for Subclade B2 and p = 16.76 % Cytb and p = 14.40 % COI for Subclade B3.

The Geomyidae underwent adaptive radiation during the Cenozoic, resulting in all of the current genera (Álvarez-Castañeda 2024). Our estimates of mitochondrial sequence divergence are consistent with this time frame (Figure 2, 3). Overall, our estimates revealed that the divergence between Heteromyidae and Geomyidae taxa occurred ~ 25.0 mya, placing the crown age for these taxa in the Early Miocene. Within the Geomyidae, estimated divergence times are ~ 14.84 mya for the tribe Thomomyini (Clade A) and ~ 10.82 mya for the Geomyini (Clade B), both of which fall between the Hemingfordian and Barstovian stages of the North American Land Mammal Ages (NALMA) scheme (Wood *et al.* 1941). Generic-level divergence times range from the Middle Miocene (~ 14 mya) to the Early-Pliocene (~ 4 mya), while most species-level divergence times fall within the Late Pliocene and Pleistocene (3.0 – 0.8 mya). Within *Thomomys,* divergence of the two subgenera occurred at ~ 7.29 mya for *Megascapheus* and ~ 7.23 mya for *Thomomys.* Diversification within the Geomyini began during the Late Miocene (~ 9.0 mya) and continued until the early Pliocene (~ 3.7 mya; Figure 2, 3).

Discussion

The use of different date sets – notably the use of different genetic markers – to examine the taxonomy and systematics of the Geomyidae has made it challenging to develop a comprehensive understanding of diversification within this family. For example, reviews of the different genera of geomyids have tended to employ different combinations of mitochondrial and nuclear genetic markers (Demastes *et al.* 2002, 2003; Hafner *et al.* 2009; Mathis *et al.* 2013a, 2013b; Spradling *et al.* 2016; Bradley *et al.* 2023), with the result that data cannot easily be compared across studies. The marker that has been most commonly used across analyses is the mitochondrial Cytb locus and for this reason we have focused our analyses on this gene. Although Cytb tends to reveal relatively low levels of differentiation (*i. e.*, small p-distances) between species, many of these distinctions are supported by data from other genes that serve to validate the separation of species. Here, monophyly was used to establish the degree of differentiation between distinct phylogenetic units; the resulting values for differentiation at the Cytb locus were then used to set boundaries between genera and subgenera, to provide a quantitative basis for distinguishing between taxonomic units at these levels. When possible, these criteria were supplemented by other data sets with potential diagnostic value, including a) time since divergence (estimated ages of clades), b) strength of support (*e. g.*, bootstrap values) for different units, c) genetic distances among other, established taxonomic units within the Geomyidae, and e) morphological

Table 2. Pairwise percentage of genetic differences based on cytochrome oxidase subunit 1 (COI) across genera and subgenera in the family Geomyidae.

		1	2	3	4	5	6	7	8	9	10
1	*Cratogeomys*										
2	*Pappogeomys*	13.99%									
		12.95-15.14									
3	*Zygogeomys*	14.73%	14.46%								
		13.65-16.02	14.27-14.64								
4	*Orthogeomys*	14.59%	15.51%	14.56%							
		13.71-16.10	15.35-15.67	14.04-15.09							
5	*Heterogeomys*	15.09%	13.56%	16.05%	14.46%						
	(subgenus)	14.25-16.34	12.82-14.18	15.55-16.37	14.05-14.89						
6	*Macrogeomys*	15.98%	14.78%	15.18%	15.24%	10.44%					
	(subgenus)	15.04-17.10	14.18-15.59	14.21-16.12	13.99-16.06	10.04-10.96					
7	*Geomys*	16.23%	15.69%	15.89%	15.72%	17.41%	17.41%				
	(west Mississippi)	15.23-17.74	15.03-16.32	15.29-16.43	15.22-16.32	16.32-18.41	16.32-18.41				
8	*Geomys*	16.31%	14.70%	15.43%	15.48%	15.47%	15.47%	14.66%			
	(east Mississippi)	15.29-16.71	14.31-15.09	15.35-15.51	15.41-15.54	14.96-15.87	14.96-15.87	13.67-15.93			
9	*Megascapheus*	18.32%	18.28%	14.57%	18.20%	18.62%	18.62%	18.25%	18.43%		
	(subgenus)	16.97-19.86	17.36-19.17	12.24-16.43	17.49-19.44	17.86-19.66	17.86-19.66	16.72-19.43	17.75-19.32		
10	*Thomomys*	18.21%	17.73%	18.05%	17.86%	18.17%	18.17%	17.52%	17.42%	15.46%	
	(subgenus)	16.91-19.11	17.16-18.33	17.51-18.78	17.29-18.34	17.25-18.91	17.25-18.91	16.66-18.54	16.58-17.88	8.34-19.49	
11	outgroup	20.00%	20.41%	19.50%	20.22%	20.07%	20.07%	20.34%	20.04%	20.11%	19.97%
		17.62-21.76	19.17-21.83	17.97-20.40	18.58-21.32	18.58-21.88	18.58-21.88	18.33-22.41	18.85-21.31	18.13-21.78	17.80-22.41

differences reported in the literature. Based on these analyses, we suggest that a formal revision of generic-level differentiation within this family is warranted.

Evidence for monophyly. Our phylogenetic analyses provided clear evidence of the monophyly of the ten terminal taxa depicted in Figure 1. With the exception of the distinct eastern and western clades of *Geomys* depicted in this figure, all other groups have been recognized previously at the generic or sub-generic levels (Demastes *et al.* 2002; Hafner *et al.* 2004, 2005, 2008; Sudman *et al.* 2006; Chambers *et al.* 2009; Mathis *et al.* 2013a, 2013b, 2014; Spradling *et al.* 2016; Álvarez-Castañeda *et al.* 2017; Bradley *et al.* 2023). In our analyses, both CytB and COI sequence data provided strong support for these monophyletic units, with 100% support being provided by one or more of the following metrics: neighbor-joining (NJ), unweighted pair group method with arithmetic mean (UPGMA), maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI; Figure 1). Monophyly within the genus *Geomys* has also been documented through the use of three combined genetic regions: the nuclear gene Rbp3, ribosomal RNA (12S rRNA), and mitochondrial DNA (Chambers *et al.* 2009). These findings align with our analysis, which includes all genera in the family and incorporates the Heteromyidae as an external group. Thus, both our data and those from previous studies indicate that the taxonomic units in Figure 1 are monophyletic. In the case of *Thomomys*, it had previously been reported based on nuclear genes (seven non-coding nuclear sequence loci) that the monophyly of the species within this genus was not wholly resolved since three of the four named species within the subgenus *Thomomys* were found to be monophyletic (Belfiore *et al.* 2008). Our analysis of mitochondrial genes supports the findings of the previously mentioned study regarding the two clades, *Megascapheus* and *Thomomys*. Belfiore *et al.* (2008) proposed that these subgenera originated approximately 5 million years ago (Ma). However, our analysis estimates the origin of these clades to be around 7 Ma. Our estimate aligns with the fossil record for Thomomyines, which date to the middle Hemphillian period (NALMA), also approximately 7 Ma, according to Shotwell (1967) and Tedford *et al.* (2004).

Genetic differentiation between taxa. At the species level, the least genetic differentiation (*i. e.*, smallest p-distance) evident in our data occurred between *Cratogeomys tylorhinus* and *C. fumosus*, which were separated by an average *p*-distance of 2.35%. Despite their limited divergence, these taxa have been recognized as distinct species based on mtDNA and nDNA analyses (Hafner *et al.* 2004). Within *Geomys*, Sudman *et al.* (2006) and Bradley *et al.* (2023) employed an estimated percent divergence of ~ 6 % to distinguish between species; this same level of divergence has been applied to Cytb data from other genera of Geomyidae that also included analyses of nuclear markers, karyotypes, and morphology (*e. g.*, *Pappogeomys*: Demastes *et al.* 2003; Hafner *et al.* 2009; *Orthogeomys* and *Heterogeomys*: Spradling *et al.* 2016; *Thomomys*: Álvarez-Castañeda 2010; Trujano-Álvarez and Álvarez-Castañeda 2013; Mathis *et al.* 2013a). Our analyses are generally consistent with this 6 % criterion, although several exceptions are evident. One is the small p-distance between C. *tylorhinus* and *C. fumosus* noted

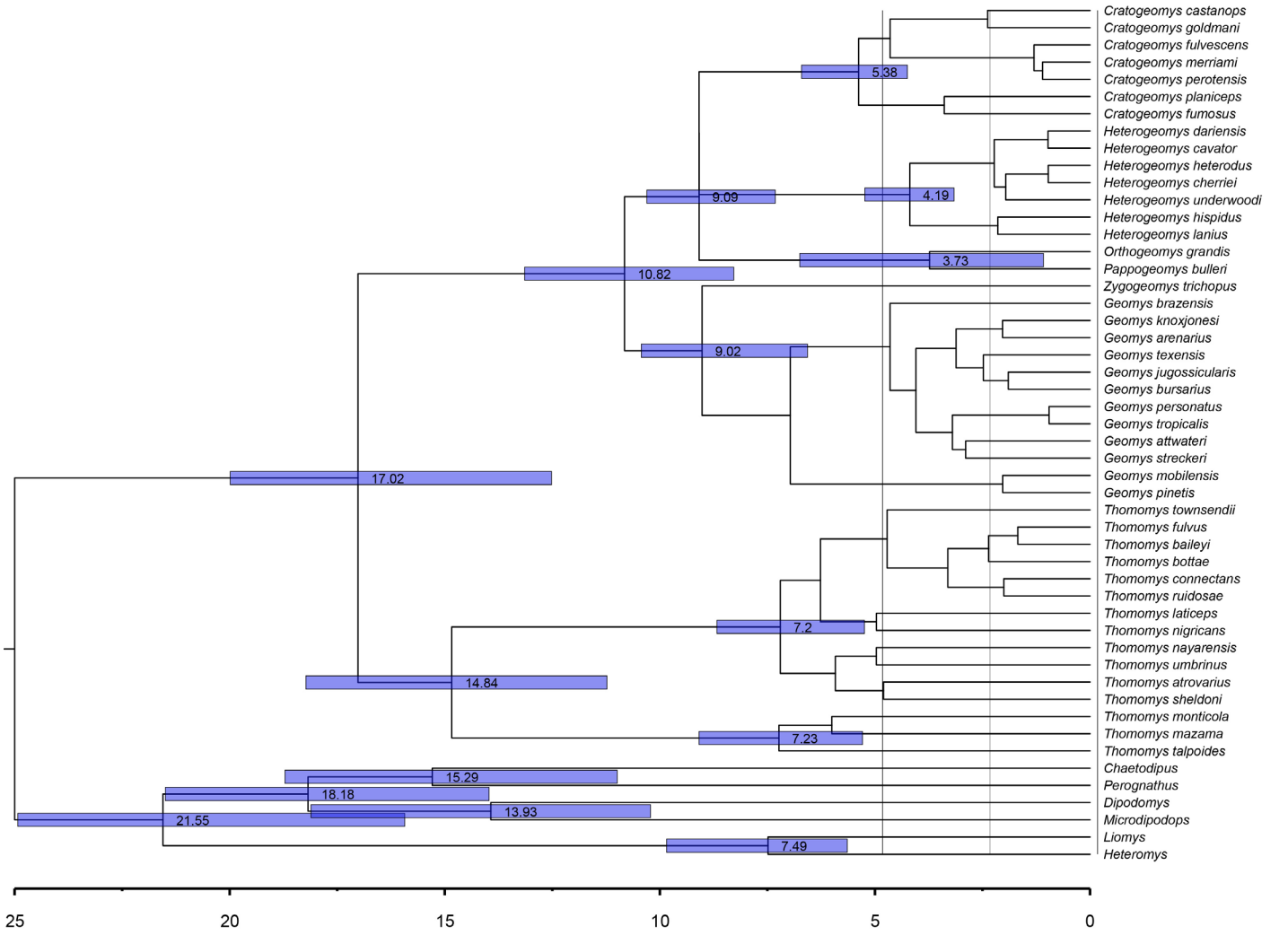


Figure 2. Calibrated maximum clade credibility tree of the Geomyidae and Heteromyidae using Cytb. Node labels include divergence time estimates in millions of years. Horizontal bars show the 95 % highest posterior density intervals surrounding each estimate. Calibration points were obtained from a Bayesian divergence dating analysis for the Heteromyidae using multigenes data (Hafner *et al.* 2007).

above. At the other extreme, differentiation with *Thomomys townsendi* ($p = 9.62$ %) and *T. talpoides* ($p = 14.61$ %) is greater than average values reported for all other species. Although use of p-distances alone to distinguish species is somewhat controversial, revisions based primarily on this metric have been conducted for geomyids, with minimal morphological information used to diagnose species of *Geomys* (Baker *et al.* 1989; Block and Zimmerman 1991; Jolley *et al.* 2000; Sudman *et al.* 2006; Chambers *et al.* 2009; Bradley *et al.* 2023) and *Thomomys* (Álvarez-Castañeda 2010; Trujano-Álvarez and Álvarez-Castañeda 2013; Álvarez-Castañeda *et al.* 2017; Bradley *et al.* 2023). While Patton 2005Beauchamp-Martin *et al.* (2019) do not recognize *Thomomys fulvus* as a valid species based on *p*-distance values, Bradley *et al.* (2023) not only accept this species but use similar criteria to recognize three other species of *Thomomys* (*T. baileyi, T. connectens,* and *T. ruidosae*). Regardless of whether p-distances alone are considered sufficient for distinguishing species, the range of values for this metric between currently recognized species of geomyids suggests that a species-level revision of these animals is warranted.

At the generic level, our analyses of Cytb sequences indicate that *Pappogeomys* and *Orthogeomys* are sibling taxa. These genera, which have long been recognized as distinct based on morphological characteristics (Hall and Kelson 1959; Russell 1968a; Hall 1981; Álvarez-Castañeda 2024), have Cytb a *p*-distance of 13.03 %, suggesting that this degree of differentiation may provide a basis for defining distinct genera. Based on COI sequences, the most closely related genera are *Pappogeomys* and *Cratogeomys*, which are separated by a *p*-distance of 14 %, providing a potential baseline divergence value for this gene. Although morphological analyses were not conducted as part of this study, information obtained from the literature suggests that with the exception of the east-west split within *Geomys*, all monophyletic groups reported here (Figure 1) have been recognized previously at the generic or subgeneric levels and that each is associated with a diagnostic description that can be clearly used for identification purposes (Merriam 1895; Nelson and Goldman 1929, 1934;Hall and Kelson 1959; Anderson 1966, 1972; Russell 1968a, b; Hall 1981; Álvarez-Castañeda 2024). Thus, available information indicates that

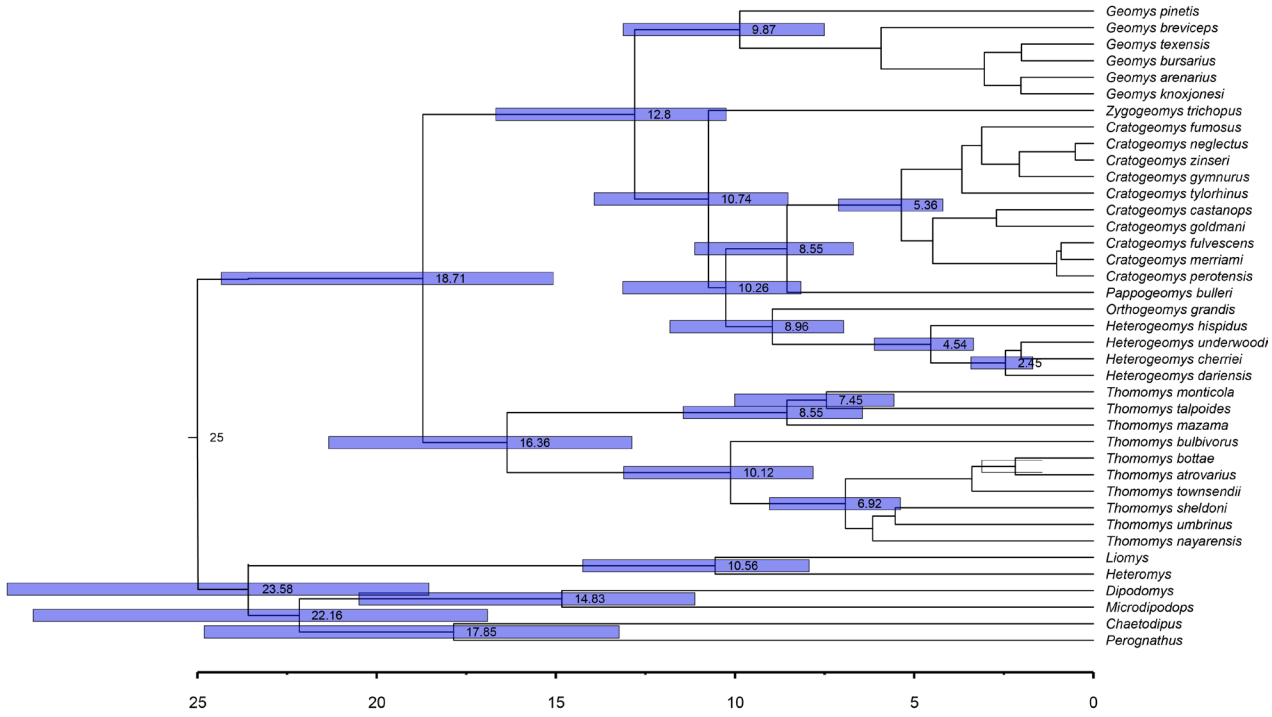


Figure 3. Calibrated maximum clade credibility tree of the Geomyidae and Heteromyidae using COI. Node labels include divergence time estimates in millions of years. Horizontal bars show the 95 % highest posterior density intervals surrounding each estimate. Calibration points were obtained from a Bayesian divergence dating analysis for the Heteromyidae using multigenes data (Hafner *et al.* 2007).

the taxonomic units revealed by analyses of Cytb and COI are robust and should be recognized as distinct.

Review of geomyid taxonomy at the generic level. Our phylogenetic analyses revealed a clear separation between the two standardly recognized sub-clades of geomyids, the tribes Geomyini and Thomomyini (Russell 1968a; Appendix 1).

Tribe Thomomyini. Our analyses indicate that the tribe Thomomyini (our Clade A) is composed of two reciprocally monophyletic clades, each related to one of the subgenera of *Thomomys* proposed by Thaeler (1980), namely *Megascapheus* and *Thomomys* (Patton and Smith 1981, 1989). *Megascapheus* was proposed by Elliot (1903) but was not recognized by Russell (1968a) or Hall (1981). Our data reveal a clear genetic separation between the proposed sub-genera (p-distance =19.2% for Cytb and 15.46% for COI). Morphologically, *Megascapheus* species can be distinguished from *Thomomys* based on a variety of cranial traits (Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024); cytogenetically, the number of chromosomes also differs markedly. Thus, overall, our analyses support recognition of *Megascapherus* and *Thomomys* as distinct taxonomic units. The degree of genetic differentiation detected between these taxa suggests that they likely should be recognized as distinct genera. concern the skull and teeth, structures normally used as diagnostic attributes at the genus level.

Tribe Geomyini. The Geomyini (our Clade B) was proposed by Russell (1968a) and includes six genera: *Cratogeomys*, *Geomys, Heterogeomys* (subgenus *Heterogeomys* and *Macrogeomys*), *Orthogeomys, Pappogeomys,* and *Zygogeomys*. This scheme has been accepted since it was first proposed, although there have been subsequent modification at the generic and sub-generic levels (Russell 1968a, b; Hall 1981; Spradling *et al.* 2016). Our analyses indicate that the Genomyini are divided into three reciprocally monophyletic sub-clades. The first of these (our subclade B1) contains the genera *Cratogeomys*, *Pappogeomys, Orthogeomys*, and *Zygogeomys. Cratogeomys* has been considered as a subgenus of *Pappogeomys* (Russell 1968a, b) but our analyses support those of Demastes *et al.* (2003) in suggesting that these are distinct genera. Our Cytb analyses suggest that *Pappogeomys* is sibling to *Orthogeomys;* this is in contrast to previous work that placed *Orthogeomys* closer to *Zygogeomys* (Russell 1968a). Results from other studies indicate that *Pappogeomys* and *Orthogeomys* are clearly morphologically different (Merriam 1895; Russell 1968a; Honeycutt and Williams 1982; DeWalt *et al.* 1993; Demastes *et al.* 2002, 2003; Spradling *et al.* 2016; Nelson and Goldman 1934; Álvarez-Castañeda 2024) and thus *p*-distances between these taxa (p = 13 % for Cytb and 14 % for COI) may provide useful metrics for evaluation the degree of genetic differentiation between other putative genera of geomyids. The final genus in this sub-clade, *Zygogeomys,* contains only one extant species that is endemic to Michoacán, Mexico; these animals are clearly morphologicall distinct from other members of the sub-clade (Merriam 1895, Russell 1968a, Álvarez-Castañeda 2024), thereby supporting recognition of *Zygogeomys* as a separate genus.

The second sub-clade of Geomyini (our sub-clade B2) includes the genus *Heterogeomys,* which consists of two

Table 3. Average and range of genetic differences based on Cytb and COI within each of the species examined in the family Geomyidae. *Only one individual was recorded.

Species	Cytb Average (Min – Max)	COI Average (Min – Max)
Cratogeomys castanops	1.42% (0.09 - 3.07)	2.45% (0.00 - 4.07)
Cratogeomys fulvescens	0.39% (0.00 - 0.61)	0.48% (0.13 - 0.78)
Cratogeomys fumosus	1.87% (1.14 - 2.46)	*
Cratogeomys goldmani	2.75% (0.49 - 5.15)	0.03% (0.01 - 0.04)
Cratogeomys gymnurus	2.81% (0.44 - 4.04)	*
Cratogeomys merriami	2.58% (0.00 - 4.74)	0.99% (0.00% 1.82)
Cratogeomys neglectus	0.72% (0.26 - 4.38)	*
Cratogeomys perotensis	1.26% (0.00 - 2.02)	0.93% (0.06 - 1.49)
Cratogeomys planiceps	0.94% (0.53 - 1.23)	
Geomys arenarius	3.34% (0.00 - 6.24)	*
Geomys attwateri	2.43% (1.32 - 3.84)	
Geomys bursarius	3.55% (0.00 - 5.61)	*
Geomys jugossicularis	1.83% (0.18 - 2.77)	
Geomys knoxjonesi	2.12% (0.09 - 2.72)	*
Geomys personatus	0.66% (0.00 - 0.96)	
Geomys mobilensis	0.31% (0.00 - 0.56)	
Geomys pinetis	2.02% (0.18 - 3.05)	0.04% (0.00 - 0.06)
Geomys streckeri	0.43% (0.00 - 0.89)	
Geomys texensis	1.44% (0.00 - 2.89)	0.58% (0.58 - 0.58)
Geomys tropicalis	1.05% (0.52 - 1.57)	
Orthogeomys cavator	2.89% (2.89 - 2.89)	
Orthogeomys cherriei	0.72% (0.26 - 1.23)	*
Orthogeomys dariensis	0.25% (0.00 - 0.53)	*
Orthogeomys grandis	*	3.98% (2.26 - 5.14)
Orthogeomys heterodus	0.24% (0.00 - 0.75)	
Orthogeomys hispidus	0.39% (0.00 - 0.79)	1.42% (0.34 - 2.74)
Orthogeomys lanius	*	
Orthogeomys underwoodi	1.34% (0.76 - 1.76)	*
Pappogeomys bulleri	5.83% (0.60 - 8.01)	3.89% (0.00 - 5.26)
Thomomys atrovarius	4.59% (0.35 - 5.88)	9.02% (2.01 - 4.88)
Thomomys bottae	3.14% (0.96 - 4.74)	5.65% (0.00 - 4.50)
Thomomys bulbivorus		*
Thomomys fulvus	4.58% (0.20 - 6.83)	
Thomomys laticeps	3.30% (2.02 - 4.24)	
Thomomys mazama	0.58% (0.09 - 0.88)	*
Thomomys monticola	0.06% (0.00 - 0.09)	*
Thomomys nayarensis	0.61% (0.00 - 1.23)	0.65% (0.65 - 0.65)
Thomomys nigricans	1.74% (0.20 - 2.61)	
Thomomys sheldoni	4.47% (0.79 - 6.23)	4.21% (3.44 - 5.39)
Thomomys talpoides	14.6% (6.32 - 16.84)	5.27% (0.00%- 9.91)
Thomomys townsendii	9.62% (9.62 - 9.62)	*
Thomomys umbrinus	3.89% (0.18 - 5.90)	*
Zygogeomys trichopus	*	*

recognized subgenera, *Heterogeomys* and *Macrogeomys* (Spradling *et al.* 2016). *Heterogeomys* was previously considered a sub-genus of *Orthogeomys* (Russell 1968a; Hall 1981; Patton 2005), but our analyses indicate that the former is clearly distinct from *Orthogeomys*. Although relationships among the species in *Heterogeomys* and *Macrogeomys* have been reviewed previously (Hafner 1991; Sudman and Hafner 1992), it appears that no detailed analysis has been conducted at the level of these subgenera. Differentiation of *Heterogeomys* and *Macrogeomys* is strongly supported by our analyses (Figure 1). Although p-distances between these taxa (11.09 % for Cytb; 10.44 % for COI) are somewhat lower than those reported for *Pappogeomys* and *Orthogeomys*, the strong support for the monophyly of these units coupled with documented morphological differences between them (Russell 1968a; Hall 1981) lead us to support their recognition as distinct sub-genera.

The final sub-clade (our sub-clade B3) includes all species of *Geomys sensu lato*. While our analyses support the monophyly of this genus, they also reveal the presence of two distinct, reciprocally monophyletic lineages that correspond to the eastern and western sides of the Mississippi River (Figure 1). The clade occurring to the east of the Mississippi has been recognized previously as part of the *G. pinetis* species complex (Russell 1968a; Penney and Zimmerman 1976; Sudman *et al.* 2006). In contrast, the clade to the west of the Mississippi includes the *bursarius* and *breviceps* species groups (Davis 1940; Hall 1981; Sudman *et al.* 2006). The two lineages display marked variation in cranial morphology (Russell 1968a; Penney and Zimmerman 1976; Sudman *et al.* 2006), lending further support to the apparent differentiation of these animals. These geographically distinct lineages are characterized by genetic distances (p = 16.72 % for Cytb and 14.66 % for COI) that are larger than those reported here for pairs of established genera (*e. g.*, *Pappogeomys-Orthogeomys*), suggesting that their inclusion within the single genus *Geomys* should be reconsidered and the more derived, western lineage potentially elevated to a distinct genus.

Geography of diversification within Geomyidae. No subgenera have been recognized in *Geomys*, but the analyses in the present study reveal a large *p*-distance between members of this genus from different sides of the Mississippi

Table 4. Average and range of genetic differences based on Cytb and COI within each of the taxa examined in the family Geomyidae. *Only one species has been sequenced. ** Only two species have been sequenced.

Taxa	Cytb Average (Min – Max)	COI Average (Min – Max)
Cratogeomys (genus)	11.81% (4.64 - 15.08)	9.45% (2.59 -11.96)
Pappogeomys (genus)	*	*
Zygogeomys (genus)	*	*
Orthogeomys (genus)	*	*
Heterogeomys (genus)	10.62% (3.91 – 17.67)	8.62% (6.84 - 10.96)
Heterogeomys (subgenus)	7.01%**	1.09%**
Macrogeomys (subgenus)	7.92% (3.91 -11.31)	7.50% (6.77 - 8.69)
Geomys (genus)	13.58% (3.24 – 19.03)	11.76% (6.93 - 15.93)
Geomys (W Mississippi)	12.40% (4.90 – 19.03)	10.05% (6.93 – 13.34)
Geomys (E Mississippi)	*	*
Thomomys (genus)	16.09% (4.21 – 21.22)	14.40% (6.28 - 19.49)
Megascapheus (subgenus)	14.01% (4.21 - 18.21)	12.69% (6.29 - 15.61)
Thomomys (subgenus)	17.71% (7.01 – 20.08)	14.46% (12.74 – 16.12)

River. Animals from west of the river include the *bursarius* and *breviceps* species groups, while animals from east of the river include species in the *pinetis* group (Davis 1940; Hall 1981; Sudman *et al.* 2006). More broadly, patterns of evolutionary diversification differ markedly between the two recognized tribes of geomyids. While the Thomomyini contains two genera, the Geomyini consists of at least seven genera. The Geomyidae are typically considered to be a fast-evolving group, with much of their diversity emerging during the Late Miocene. This coincides with the expansion of open grass-dominated habitats during the Cenozoic (Strömberg 2011; Anderman *et al.* 2022), which may have facilitated diversification of these herbivorous rodents. Divergence within the Geomyidae is thought to have been driven by multiple factors, including fluctuations in climatic conditions and their impacts on vegetation, notably the relative expansions and contractions of forests versus grasslands (Castañeda-Rico *et al.* 2024). At the same time, geographic barriers have no doubt played a role in this dynamic, as has been suggested for the role of the Trans-Mexican Volcanic Belt (TMVB) in determining the distributional limits of multiple genera of geomyids and generating a unique habitat for species in the genera *Pappogeomys* and *Zygogeomys*. It seems likely that the Mississippi River has also functioned as an important geographic barrier, particularly within *Geomys,* which is the only currently recognized genus of pocket gophers to cross this river. Accordingly, it is not surprising that there are marked genetic and morphological differences between members of this genus located on the western versus eastern sides of this major riverine barrier.

Concluding thoughts. Based on our findings that members of the genus *Geomys* form two genetically distinct lineages that are separated by the Mississippi River, we suggest that formal revision of the genus is warranted, with attention to whether differences between these lineages are sufficient to justify recognition of each as a distinct genus. Our analyses are based on sequence data from two mitochondrial locus indicate that levels of genetic differentiation (p-distances) are greater than those for other pairs of genera within the Geomyidae. These differences are also supported by previous studies describing morphological differences between these lineages. We assert that, given these differences, the two lineages should be formally distinguished using taxonomic categories recognized by the ICZN (1999). We believe that elevation of the western lineage to genus status is more appropriate than description of the two lineages as species groups or complexes as the latter designations lack ICZN (1999) oversight and often add unnecessary complexity to efforts to resolve mammalian taxonomy (Tate 1933; Voss *et al.* 2014). At the same time, we recognize that an integrated approach –one that makes use of multiple data sets– is critical when diagnosing new taxonomic units. A formal revision of genera within the Geomyidae, in particular evaluation of our proposal that *Geomys* be divided into two genera, will benefit from examination of nuclear sequence data as well as empirical evaluation of apparent morphological differences between lineages. More generally, significant revision is needed for other portions of the Geomyidae. Although our analyses have focused on generic level differences within this family, revision at other levels is also needed, such as a revision of species-level differentiation with Thomomys. We hope the analyses included here will provide the foundation for a more extensive and comprehensive revision of the taxonomy of the family Geomyidae. Given the ecological and evolutionary importance of these animals, a thorough understanding of their taxonomic diversity should generate critical insights into numerous aspects of mammalian biology.

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Appendix 1

SYSTEMATICS

We propose that pocket gophers in the family Geomyidae be classified into nine genera, as defined, diagnosed, and discussed in the following generic accounts. For the sake of reference completeness, the literature cited provides the full citations for each of the generic-level names (Merriam 1895; Elliot 1903).

Tribe Thomomyini (Clade A)

Genus *Megascapheus* Elliot 1903

1903. *Megascapheus* Elliot, Field Columb. Mus., Publ. 76, Zool. Ser., 3(11):190. Type species. *Diplostoma bulbivorum* (Richardson, 1829).

Content. Thirteen allopatric species of *Megascapheus* are recognized: *Megascapheus atrovarius* (Allen, 1898), *Megascapheus baileyi* (Merriam, 1901), *Megascapheus bottae* (Eydoux and Gervais, 1836), *Megascapheus bulbivorus* (Richardson, 1829), *Megascapheus connectens* (Hall, 1936), *Megascapheus fulvus* (Woodhouse, 1852), *Megascapheus laticeps* (Baird, 1855), *Megascapheus nayarensis* (Mathis *et al.,* 2013), *Megascapheus nigricans* (Rhoads, 1895), *Megascapheus ruidosae* (Hall, 1932), *Megascapheus sheldoni* (Bailey, 1915), *Megascapheus townsendii* (Bachman, 1839), and *Megascapheus umbrinus* (Richardson, 1829).

Etymology. The name *Megascapheus* is derived from the Greek *Mega*, meaning "great" and *skapherus, "*a digger": the great digger (Jaeger 1955).

Diagnosis. The species in the genus *Megascapheus* can be distinguished from those in the genus *Thomomys* by having the upper incisors procumbent and their root between the fourth upper premolars and the first upper molars; sphenoidal fissure open; angular process continuous, with a well-developed flange along the ventral ramus side; rostrum heavy; base of the first lower premolars inclined anteriorly; infraorbital canal openings anterior to the incisive foramina; anterior enamel plate of the first lower premolars not recurved; anterior enamel plate of the first lower premolars narrow and broadly separated from the lateral enamel plate on the lingual side; chromosome number of living forms from 74 to 82 (Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024).

Distribution. *Megascapheus* ranges from southern Oregon, Idaho, and Colorado southward through Michoacán, State of Mexico, Mexico City, Puebla, and Veracruz and eastward through New Mexico, Texas, Coahuila, Nuevo Leon, San Luis Potosí, and Veracruz.

Comments. *Megascapheus* and *Thomomys* were considered two subgenera of *Thomomys*, with clear morphological differences. Morphological and genetic data support considering these differences sufficient at the genus level. The subspecies of *M. fulvus* should be reviewed, increasing the number of sequences of the recognized subspecies to carry out a detailed analysis. The nomenclature used in the genus is supported in the studies of Hall and Kelson (1959), Anderson (1966, 1972), Patton and Dingmen (1968), Thealer (1968a, b, 1972, 1977, 1980), Hoffmeister (1969, 1986), Patton (1973, 1993, 2005), Thaeler and Hinesley (1979), Patton and Smith (1981, 1990), Patton *et al.* (1984), Álvarez-Castañeda (2010), Hafner *et al.* (2011), Trujano-Álvarez and Álvarez-Castañeda (2013), Mathis *et al.* (2013a, 2013b, 2014), Álvarez-Castañeda *et al.* (2017), and Bradley *et al.* (2023).

Morphological difference between *Megascapheus* in relation to *Thomomys* are: the upper incisors procumbent and their root between the fourth upper premolars and the first upper molars *vs* upper incisors not procumbent and their root above the fourth upper premolars; sphenoidal fissure open *vs* closed (except for some specimens of *T. clusius*); angular process continuous, with a well-developed flange along the ventral ramus side *vs* not continuous, with a weakly developed flange along the ventral ramus side; rostrum heavy vs rostrum slender; base of the first lower premolars inclined anteriorly *vs* nearly perpendicular to the occlusal surface of the toothrow; infraorbital canal openings anterior to the incisive foramina *vs* directly above or slightly posterior to the incisive foramina; anterior enamel plate of the first lower premolars not recurved *vs* recurved, frequently forming a shallow re-entrant angle; anterior enamel plate of the first lower premolars narrow and broadly separated from the lateral enamel plate on the lingual side, *vs* only slightly separated from the posterior enamel plate (rarely continuous), with the lateral enamel plate on the lingual side; chromosome number of living forms from 74 to 82 *vs* from 40 to 60 (Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024).

Megascapheus atrovarius (Allen, 1898)

(Southern pocket gopher, tuza de Sinaloa)

1898. *Thomomys atrovarius* (J. A. Allen), Bull. Amer. Mus. Nat. Hist., 10:148. Type locality "Tatemales (near Rosario), Sinaloa".

2024. *Megascapheus atrovarius*: (this study).

1. *M. a. atrovarius* (Allen, 1898). For type locality see above. Range from the central Sinaloa coast south through northwestern Jalisco.

2. *M. a. parviceps* (Nelson and Goldman, 1934). Type locality "Chacala, 3000 ft., Durango". Known only from central and northeastern Sinaloa and western Durango.
3. *M. a. simulus* (Nelson and Goldman, 1934). Type locality "Alamos, 1200 ft., Sonora". Known only from southeastern Sonora and northeastern Sinaloa.
4. *M. a. sinaloae* (Merriam, 1901). Type locality "Altata, Sinaloa". Range in coastal central and northern Sinaloa.

Megascapheus baileyi (Merriam, 1901)

Southwestern Texas pocket gopher, tuza del suroeste de Texas

1901. *Thomomys baileyi* Merriam, Proc. Biol. Soc. Washington, 14:109. Type locality "Sierra Blanca, Hudspeth Co., Texas".

2024. *Megascapheus baileyi*: (this study).

1. *M. b. actuosus* (Kelson, 1951). Type locality " Corona, Lincoln Co., New Mexico". Known only from central New Mexico.
2. *M. b. analogus* (Goldman, 1938a). Type locality " Sierra Guadalupe, about 12 mi. S General Cepeda, Coahuila". Range in southeastern Coahuila, southwestern Coahuila, and Nuevo León.
3. *M. b. baileyi* (Merriam, 1901). For type locality see above. Known only from type locality.
4. *M. b. confinalis* (Goldman, 1936). Type locality " 35 mi. E Rock Springs, 2450 ft., Texas". Known only from Sutton County, southwestern Texas.
5. *M. b. cultellus* (Kelson, 1951). Type locality " Halls Peak, Mora Co., New Mexico". Known only from Mora County, northeastern New Mexico.
6. *M. b. guadalupensis* (Goldman, 1936). Type from McKittrick Canyon, 7800 ft., Guadalupe Mts., Texas." Known only from Guadalupe Mountains New Mexico and Texas.
7. *M. b. humilis* (Baker, 1953). Type locality " 3 mi. W Hda. San Miguel, 2200 ft., Coahuila". Known only from northern Coahuila.
8. *M. b. lachuguilla* (Bailey, 1902). Type locality " arid foothills near El Paso, El Paso Co., Texas". Range in southern New Mexico and western Texas.
9. *M. b. limitaris* (Goldman, 1936). Type locality "4 mi. W Boquillas, Brewster Co., Texas". Known only from southwestern Texas.
10. *M. b. limpiae* (Blair, 1939). Type locality " Limpia Canyon, 1 mi. N Fort Davis, 4700 ft., Jeff Davis Co., Texas". Known only from Jeff Davis County, western Texas.
11. *M. b. opulentus* (Goldman, 1935). Type locality " Las Palomas, on the Rio Grande, Sierra Co., New Mexico". Known only from Sierra County, central New Mexico.
12. *M. b. pectoralis* (Goldman, 1936). Type locality "Vicinity of Carlsbad Cave, Carlsbad Cave National Monument, Eddy Co., New Mexico". Known only from Carlsbad Cave National Monument.
13. *M. b. pervagus (*Merriam, 1901). Type locality "Española, Rio Arriba Co., New Mexico". Known from northern New Mexico.
14. *M. b. retractus* (Baker, 1953). Type locality "Fortin, 3300 ft., 20 mi. N, 2 mi. E San GerOnimo, Coahuila". Known only from northern Coahuila.
15. *M. b. robertbakeri* (Beauchamp-Martin *et al.* 2019). Type locality "2.5 mi. E McCamey, Upton County, Texas". Known only from southern-central Texas.
16. *M. b. scotophilus* (Davis, 1940). Type locality "1 1/2 W Bat Cave, Sierra Diablo, Hudspeth Co., Texas. Known only from Sierra Diablo Texas.
17. *M. b. spatiosus* (Goldman, 1938). Type locality "Alpine, 4500 ft., Brewster Co., Texas". Known only from Brewster County, western Texas.
18. *M. b. sturgisi* (Goldman, 1938a). Type locality "Sierra del Carmen, 6000 ft., Coahuila". Range from central Coahuila to northwestern Coahuila.
19. *M. b. texensis* (Bailey, 1902). Type locality "head of Limpia Creek, 5500 ft., Davis Mts., Jeff Davis Co., Texas". Known only from southwestern Texas.
20. *M. b. tularosae* (Hall, 1932). Type locality "Cook Ranch, ½ mi. W Tularosa, Otero Co., New Mexico". Known only from Tularosa are in New Mexico.
21. *M. b. villai* (Baker, 1953). Type locality "7 mi. S, 2 mi. E Boquillas, 1800 ft., Coahuila". Known only from type locality.

Megascapheus bottae (Eydoux and Gervais, 1836)

Botta's pocket gopher, tuza del norte

1836. *Oryctomys* (*Saccophorus*) *bottae* Eydoux and Gervais, Mag. de Zool., Paris, 6:23. Type locality "Coast of California"; name applied by Baird (Proc. Acad. Nat. Sci. Philadelphia, 7:335) to the gopher occurring in vie. Monterey.

1966. *Thomomys bottae*: Anderson, Syst. Zool., 15:192.

2024. *Megascapheus bottae*: (this study).

1. *M. b. bottae* (Eydoux and Gervais, 1836). For type locality see above. Range in western coast of California from San Francisco Bay south through Ventura County.
2. *M. b. mewa* (Merriam, 1908). Type locality "Raymond, Madera Co., California". Known only from Maderas County, central California.
3. *M. b. navus* (Merriam, 1901). Type locality "Red Bluff, Tehama Co., California". Known only from Tehama County, northern California.
4. *M. b. pascalis* (Merriam, 1901). Type locality "Fresno, San Joaquin Valley, Fresno Co., California". Known only from Fresno County, central California.

Megascapheus bulbivorus (Richardson, 1829)

Camas pocket gopher, tuza del Valle de Camas

1829. *Diplostoma bulbivorum* Richardson, Fauna Boreali Americana, 1:206. Type locality "Banks of the Columbia River, Oregon," probably Portland, the only place near the Columbia River where it has been taken since. The type was reported as in the Hudson Bay Museum but has not been found *(fide* V. Bailey, N. Amer. Fauna, 39:40, November 15, 1915).

1855. *Thomomys bulbivorus*: Beiträge zur nähern Kenntniss der Säugethiere Russland's. St. Pétersburg Acad. Sci. Mem., 9:188.

2024. *Megascapheus bulbivorus*: (this study).

Megascapheus connectens (Hall, 1936)

(New Mexico pocket gopher, tuza de Nuevo Mexico)

1936. *Thomomys umbrinus connectens* Hall, Jour. Washington Acad. Sci., 26:296. Type locality " Clawson Dairy, 5 mi. N Albuquerque, 4943 ft., Bemalillo Co., New Mexico". Known only from Bernalillo County, New Mexico.

2024. *Megascapheus connectens*: (this study).

Megascapheus fulvus (Woodhouse, 1852)

Fulvus pocket gopher, tuza del suroeste

1852. *Geomys fulvus* Woodhouse, Proc. Acad. Nat. Sci. Philadelphia, 6:201. Type locality "San Francisco Mtn., Coconino Co., Arizona".

2010. *Thomomys fulvus*: Álvarez-Castañeda, Mol. Phylog. Evol., 54:679.

2024. *Megascapheus fulvus*: (this study).

1. *M. f. abstrusus* (Hall and Davis, 1935). Type locality " Fish Spring Valley, 2 mi. SE Tulle Peak, 7000 ft., Nye Co., Nevada". Known only from Nye County, southern Nevada.
2. *M. f. albicaudatus* (Hall, 1930). Type locality " Provo, 4510 ft., Utah Co., Utah". Known only from Utah County, central Utah.
3. *M. f. alexandrae* (Goldman, 1933b). Type locality " plain 5 mi. SW Rainbow Lodge, near Navajo Mtn., 6200 ft., Coconino Co., Arizona". Known only from Coconino County, northern Arizona.
4. *M. f. alpinus* (Merriam, 1897). Type locality " Big Cottonwood Meadows, 10,000 ft., 8 mi. SE Mt. Whitney peak, High Sierra, Inyo Co., California". Known only from Mount Whitney, Inyo County, western California.
5. *M. f. angustidens* (Baker, 1953). Type locality " Sierra del Pino, 5250 ft., 6 mi. N, 6 mi. W Acebuches, Coahuila". Known only from Sierra del Pino, Coahuila.
6. *M. f. apache* (Bailey, 1910). Type locality " Lake La Jara, 7500 ft., Jicarilla Apache Indian Reservation, New Mexico". Known only from Sandoval County, northern New Mexico.
7. *M. f. aureiventris* (Hall, 1930). Type locality " Fehlman Ranch, 3 mi. N Kelton, 4225 ft., Boxelder Co., Utah". Known only from Box Elder County, northern Utah.

8. *M. f. aureus* (Allen, 1893). Type locality " Bluff City, San Juan Co., Utah". Known only from San Juan County, southwestern Utah.
9. *M. f. basilicae* (Benson and Tillotson, 1940). Type locality " La Misi6n, 2 mi. W Magdalena, Sonora". Known only from central Sonora.
10. *M. f. birdseyei* (Goldman, 1937a). Type locality " Pine Valley Mts., 5 mi. E Pine Valley, 8300 ft, Washington Co., Utah". Restricted to Washington County, southwestern Utah.
11. *M. f. bonnevillei* (Durrant, 1946). Type locality " Fish Springs, 4400 ft., Juab Co., Utah". Known only from Juab County, western Utah.
12. *M. f. brevidens* (Hall, 1932a). Type locality " Breen Creek, 7000 ft., Kawich Range, Nye Co., Nevada". Known only from Nye County, southern Nevada.
13. *M. f. camoae* (Burt, 1937). Type locality " Camoa, Rio Mayo, Sonora". Known only from central-southern coast Sonora.
14. *M. f. canus* (Bailey, 1910). Type locality " Deep Hole, N end Smoke Creek Desert, Washoe Co., Nevada". Known only from Smoke Creek Desert, Washoe County, northwestern Nevada.
15. *M. f. catalinae* (Goldman, 1931). Type locality " Swnmerhaven, Santa Catalina Mts., 7500 ft., Pima Co., Arizona". Known only from Pima County, southern Arizona.
16. *M. f. cervinus* (Allen, 1895). Type locality " Phoenix, Maricopa Co., Arizona". Known only from Maricopa County, southwestern Arizona.
17. *M. f. cinereus* (Hall, 1932a). Type locality " West Walker River, Smiths Valley, 4700 ft., Lyon Co., Nevada". Known only from Lyon County, Nevada.
18. *M. f. collis* (Hooper, 1940). Type locality " Shuman's Ranch, 30 mi. S Grants, sec. 30, T. 6 N, R. 10 W, Valencia Co., New Mexico". Known only from Valencia County, western New Mexico.
19. *M. f. concisor* (Hall and Davis, 1935). Type locality " Pott's Ranch, 6900 ft., Monitor Valley, Nye Co., Nevada". Known only from Nye County, southern Nevada.
20. *M. f. contractus* (Durrant, 1946). Type locality " Scipio, 5315 ft., Millard Co., Utah". Known only from Millard County, western Utah.
21. *M. f. convergens* (Nelson and Goldman, 1934)". Type locality " Costa Rica Ranch, delta Sonora River, SW of Hermosillo, Sonora". Known only from the Sonora River delta, Sonora.
22. *M. f. convexus* (Durrant, 1939). Type locality " E side Clear Lake, 4600 ft., Millard Co., Utah". Known only from Millard County, western Utah.
23. *M. f. cultellus* (Kelson, 1951). Type locality " Halls Peak, Mora Co., New Mexico". Known only from Mora County, northeastern New Mexico.
24. *M. f. curtatus* (Hall, 1932a). Type locality " San Antonio, 5400 ft., Nye Co., Nevada". Known only from Nye County, southern Nevada.
25. *M. f. depressus* (Hall, 1932a). Type locality " Dixie Meadows (at S end Humboldt Salt Marsh), 3500 ft., Churchill Co., Nevada". Known only from Churchill County, western Nevada.
26. *M. f. desertorum* (Merriam, 1901). Type locality " Mud Spring, Detrital Valley, Mohave Co., Arizona". Known only from Detrital Valley, Mohave Country, southeastern Arizona.
27. *M. f. dissimilis* (Goldman, 1931). Type locality " E slope Mt. Ellen, 8000 ft., Henry Mts., Garfield Co., Utah". Known only from Garfield County, southern Utah.
28. *M. f. divergens* (Nelson and Goldman, 1934). Type locality " 4 mi. W Huachinera, 4000 ft., Rio Bavispe, Sonora". Known only from Huachinera, eastern Sonora.
29. *M. f. estanciae* (Benson and Tillotson, 1939). Type locality " La Estancia, 6 mi. N Nacori, Sonora". Known only from Nácori, eastern Sonora.
30. *M. f. fulvus* (Woodhouse, 1852). For type locality see above. Range from central Arizona to western New Mexico.
31. *M. f. fumosus* (Hall, 1932a). Type locality " Milman Ranch, Moores Creek, 19 mi. SE Millett P.O., Nye Co., Nevada". Known only from Nye County, southern Nevada.
32. *M. f. howelli* (Goldman, 1936). Type locality " Grand Junction, 4600 ft., Mesa Co., Colorado". Known only from Mesa County, western Colorado.
33. *M. f. internatus* (Goldman, 1936). Type locality " Salida, 7000 ft., Chaffee Co., Colorado". Known only from Chaffee County, southern Colorado.

34. *M. f. lacrymalis* (Hall, 1932a). Type locality " Arlemont [Chiatovich Ranch, Fish Lake Valley], 4900 ft., Esmeralda Co., Nevada". Known only from Esmeralda County, western Nevada.
35. *M. f. latus* (Hall and Davis, 1935). Type locality " Cherry Creek, 6500 ft., White Pine Co., Nevada". Known only from White Pine County, eastern Nevada.
36. *M. f. lenis* (Goldman, 1942). Type locality " Richfield, 5308 ft., Sevier Co., Utah". Known only from Sevier County, central Utah.
37. *M. f. levidensis* (Goldman, 1942). Type locality " Manti, about 5500 ft., Sanpete Co., Utah". Known only from Sanpete County, central Utah.
38. *M. f. lucrificus* (Hall and Durham, 1938)". Type locality " Eastgate, Churchill Co., Nevada". Known only from Churchill County, western Nevada.
39. *M. f. mearnsi* (Bailey, 1914). Type locality " Grays Ranch, 5000 ft., Animas Valley, Grant Co., New Mexico". Known only from the southwest corner of New Mexico.
40. *M. f. minimus* (Durrant, 1939). Type locality " Stansbury Island, Great Salt Lake, Tooele Co., Utah". Known only from Tooele County, northwestern Utah.
41. *M. f. modicus* (Goldman, 1931). Type locality " La Osa (near Mexican boundary), southern end of Altar Valley, Pima Co, Arizona". Known only from northern Sonora.
42. *M. f. morulus* (Hooper, 1940). Type locality " Bill Porter's Ranch, 8 mi. SE Paxton, Valencia Co., New Mexico". Only known of Cibola County, western New Mexico.
43. *M. f. nanus* (Hall, 1932a). Type locality " S end Belted Range, 5½ mi. NW White Rock Spring, 7200 ft, Nye Co., Nevada". Known only from Nye County, southern Nevada.
44. *M. f. nesophilus* (Durrant, 1936). Type locality " Antelope Island, Great Salt Lake, Davis Co., Utah". Known only from Antelope Island, Davis County, northern Utah.
45. *M. f. operarius* (Merriam, 1897). Type locality " Keeler, E side Owens Lake, Inyo Co., California". Ony knoen from Inyo County, eastern California.
46. *M. f. operosus* (Hatfield, 1942). Type locality " Peeples Valley, 4400 ft., 6 mi. N Yarnell, Yavapai Co., Arizona". Known only from Yavapai County, central Arizona.
47. *M. f. optabilis* (Goldman, 1936). Type locality " Coventry, 6500 ft., Montrose Co., Colorado". Known only from Montrose County, western Colorado.
48. *M. f. osgoodi* (Goldman, 1931). Type locality " Hanksville, Wayne Co., Utah". Known only from Wayne County, central Utah.
49. *M. f. paguatae* (Hooper, 1940). Type locality " ½ mi. N Cebolleta (Seboyeta P.O.), Valencia Co., New Mexico". Known only from Valencia County, western central New Mexico.
50. *M. f. peramplus* (Goldman, 1931). Type locality "Wheatfields Creek, 7000 ft. [about 27 mi. E Chin Lee], W slope Tunitcha Mts., Apache Co., Arizona". Known only from Apache County, northwestern Arizona.
51. *M. f. perditus* (Merriam, 1901). Type locality "Lampazos, Nuevo León". Range from eastern Coahuila and western Nuevo León.
52. *M. f. perpallidus* (Merriam, 1886). Type locality "Palm Springs, Riverside Co., California". Known only from Riverside County, southern California.
53. *M. f. pervagus* (Merriam, 1901). Type locality "Española, Rio Arriba Co., New Mexico". Known only from Rio Arriba and Santa Fe counties, northern New Mexico.
54. *M. f. phelleoecus* (Burt, 1933). Type locality "Hidden Forest, 8500 ft., Sheep Mts., Clark Co., Nevada". Known only from Clark County, southern Nevada.
55. *M. f. pinalensis* (Goldman, 1938b). Type locality "Oak Flat, 5 mi. E Superior, Pinal Mts., Pinal Co., Arizona". Known only from Gila County, central Arizona.
56. *M. f. planirostris* (Burt, 1931). Type locality "Zion National Park, Washington Co., Utah". Known only from Washington County, southwestern Utah.
57. *M. f. planorum* (Hooper, 1940). Type locality "1 ½ mi. SW San Mateo, Valencia Co., New Mexico". Known only from Valencia County, western-central New Mexico.
58. *M. f. powelli* (Durrant, 1955). Type locality "Hall Ranch, Salt Gulch, 8 mi. W Boulder, 6000 ft., Garfield Co., Utah". Known only from Garfield County, southern Utah.
59. *M. f. pusillus* (Goldman, 1931). Type locality "Coyote Mis., 3000 ft., Pima Co., Arizona". Known only from Pima County, southern Arizona.

60. *M. b. riparius* (Grinnell and Hill, 1936a). Type locality "Blythe, Riverside Co., California". Known only from Riverside County, southern California.
61. *M. f. robustus* (Durrant, 1946). Type locality "Orr's Ranch, 4300 ft., Skull Valley, Tooele Co., Utah". Known only from Tooele County, northwestern Utah.
62. *M. f. sevieri* (Durrant, 1946). Type locality "Swasey Spring, 6500 fl., House Mtn., Millard Co., Utah". Known only from Millard County, western Utah.
63. *M. f. solitarius* (Grinnell, 1926). Type locality "Fingerrock Wash, 5400 ft., Stewart Valley, Mineral Co., Nevada". Known only from Mineral County, western Nevada.
64. *M. f. stansburyi* (Durrant, 1946). Type locality "South Willow Creek, Stansbury Mts., 7500 ft., Tooele Co., Utah". Known only from Tooele County, northwestern Utah.
65. *M. f. subsimilis* (Goldman, 1933). Type locality "Harquahala Mt., 3000 ft., Yuma Co., Arizona". Known only from Yuma County, southwestern Arizona.
66. *M. f. tivius* (Durrant, 1937). Type locality "Oak Creek Canyon, 6 mi. E Oak City, 6000 ft., Millard Co., Utah". Known only from Millard County, western Utah.
67. *M. f. toltecus* (Allen, 1893). Type locality "Colonia Juarez, 4500 ft., Casas Grandes River, Chihuahua". Range from southern New Mexico and northern Chihuahua.

Megascapheus laticeps (Baird, 1855)

Northern California pocket gopher, tuza del norte de California

1855. *Thomomys laticeps* Baird, Proc. Acad. Nat. Sci. Philadelphia, 7:335. Type locality. Humboldt Bay, Humboldt Co., California.

2024. *Megascapheus laticeps*: (this study).

1. *M. l. agricolaris* (Grinnell, 1935). Type locality "Stralock Farm, 3 mi. W Davis, Yolo Co., California". Known only from Yolo County, California.
2. *M. l. awahnee* (Merriam, 1908). Type locality "Yosemite Valley, 4000 ft., near old Sentinel Hotel, Mariposa Co., California". Range in southern Sierra Nevada, California.
3. *M. l. detumidus* (Grinnell, 1935). Type locality "1 1/2 mi. S (town of) Pistol River, 250 ft., Curry Co., Oregon". Known only from Curry County, Oregon.
4. *M. l. laticeps* (Baird, 1855). For type locality see above. Range from Humboldt County, California north through southern Oregon.
5. *M. l. leucodon* (Merriam, 1897). Type locality "Grant Pass, Rogue River Valley, Oregon". Range in highlands around the northern Central Valley, California, and southwestern Oregon.
6. *M. l. saxatilis* (Grinnell, 1934). Type locality "1 mi. N Susan·ville, 4400 ft., Lassen Co., California". Known only from Lassen County, California.

Megascapheus nayarensis (Mathis *et al.,* 2013)

Nayarit pocket gopher, tuza del Nayar

2013. *Thomomys nayarensis* Mathis, Hafner, Hafner, and Demastes; Jour. Mamm 94:989. Type locality. 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), Nayarit, México".

2024. *Megascapheus nayarensis*: (this study).

Megascapheus nigricans (Rhoads, 1895)

California pocket gopher, tuza de Baja California

1895. *Thomomys fulvus nigricans* Rhoads; Proc. Acad. Nat. Sci. Philadelphia, 47:36. Type locality. Witch Creek, 2753 ft., 7 mi. W Julian, San Diego Co., California.

2013. *Thomomys nigricans*: Trujano-Álvarez and Álvarez-Castañeda, Zool. Jour. Linn, Soc. 168:886.

2024. *Megascapheus nigricans*: (this study).

1. *M. n. anitae* (Allen, 1898). Type locality "Santa Anita, Baja California [Sur]". Range from southern Vizcaíno Desert south to the southern tip of Baja California Peninsula.

2. *M. n. martirensis* (Allen, 1898). Type locality "La Grulla Meadow, Sierra San Pedro Mártir, 7400 ft., Baja California". Range from Sierra Juárez south through the Central Desert, Baja California.
3. *M. n. nigricans* (Rhoads, 1895). For type locality see above. Known only from southern California and northwestern Baja California.
4. *M. n. russeolus* (Nelson and Goldman, 1909). Type locality "San Angel, WSW San Ignacio, Baja California". Known only from the Vizcaíno Desert, northern Baja California Sur, and southern Baja California.

Megascapheus ruidosae (Hall, 1932)

Ruidoso pocket gopher, tuza de Ruidoso

1932. *Thomomys umbrinus ruidosae* Hall, Proc. Biol. Soc. Washington, 45:96. Type locality "Ruidoso, 6700 ft., Lincoln Co., New Mexico.

2024. *Megascapheus ruidosae*: (this study).

Megascapheus sheldoni (Bailey, 1915)

Sheldon´s pocket gopher, tuza de la Sierra Madre

1915. *Thomomys sheldoni* V. Bailey, N. Amer. Fauna, 39:93, November 15. Type locality "Santa Teresa, 6800 ft., Sierra del Nayarit, Nayarit".

2024. *Megascapheus sheldoni*: (this study).

1. *M. s. chihuahuae* (Nelson and Goldman, 1934). Type locality "Sierra Madre, 7000 ft., about 65 mi. E Batopilas, Chihuahua". Known only from the Sierra Madre Occidental highlands, Chihuahua.
2. *M. s. sheldoni* (Bailey, 1915). For type locality see above. Known only from the Sierra Madre Occidental highlands, western Durango, northeastern Nayarit, and western Zacatecas.

Megascapheus townsendii (Bachman, 1839)

Townsend´s pocket gopher, tuza de las montañas del oeste

1839. *Geomys townsendii* Bachman, Jour. Acad. Nat. Sci. Philadelphia, 8:105. Type locality. Erroneously given as "Columbia River," but probably near Nampa, Canyon Co., Idaho, where Townsend's party camped to trade with Indians, August 22, 1834 (V. Bailey, N. Amer. Fauna, 39:42, November 15, 1915).

1968. *Thomomys townsendii*: Thaler, Univ. California Pub. Zool. 86.

2024. *Megascapheus townsendii*: (this study).

1. *M. t. nevadensis* (Merriam, 1897). Type locality "Reese River Valley, 5 mi. W Austin, Lander Co., Nevada". Central-northern Nevada, southeastern Oregon, and California.
2. *M. t. townsendii* (Bachman, 1839). For type locality see above. Known only from Snake River, western Idaho, and eastern Oregon.

Megascapheus umbrinus (Richardson, 1829)

Southern pocket gopher, tuza mexicana

1829. *Geomys umbrinus* Richardson, Fauna Boreali-Americana,1:202. Type locality "southern México; probably vic. Boca del Monte, Veracruz"; type said to have come from "Cadadaguois, a town in southwestern Louisiana"; see V. Bailey, Proc. Biol. Soc. Washington, 19:3-6, January 29, 1906.

1855. *Thomomys umbrinus*: Baird, Proc. Acad. Nat. Sci. Philadelphia, 7:332.

2024. *Megascapheus umbrinus*: (this study).

1. *M. u. durangi* (Nelson and Goldman, 1934). Type locality "Durango, Durango". Range from southwestern Durango to extreme northwestern Zacatecas.
2. *M. u. goldmani* (Merriam, 1901). Type locality "Mapimi, 3800 ft., Durango". Range in central Chihuahua south through central-eastern Durango and southwestern Coahuila.
3. *M. u. intermedius* (Mearns, 1897). Type locality "summit Huachuca Mts., 9000 ft., Arizona". Range from southeastern Arizona south through Sonora and northwestern Chihuahua.
4. *M. u. umbrinus* (Richardson, 1829). For type locality see above. Range from eastern-central Zacatecas south through the Eje Neovolcánico in Veracruz.

Thomomys Wied-Neuwied, 1839

1839. *Thomomys* Wied-Neuwied, Nova Acta Phys.-Med. Acad. Caesar. Leop.-Carol., 19(pt. 1):377. Type species. *Thomomys rufescens* Wied-Neuwied, 1839.

Content. Five allopatric species of *Thomomys* are recognized: *Thomomys clusius* Coues, 1875, *Thomomys idahoensis* Merriam, 1901, *Thomomys mazama* Merriam, 1897, *Thomomys monticola* J. A. Allen, 1893, and *Thomomys talpoides* (Richardson, 1828).

Etymology. The name *Thomomys* is derived from the Greek *thomos,* meaning "pile", and *mys, "*mouse": related to the pile of earth accumulated at the entrance of its burrows.

Diagnosis. The species of the genus *Thomomys* can be distinguished from those of *Megascapheus* by having the upper incisors no procumbent and their root above the fourth upper premolars; sphenoidal fissure close (except for some specimens of *T. clusius*); angular process not continuous, with a weakly-developed flange along the ventral ramus side; rostrum slender; base of the first lower premolars nearly perpendicular to the occlusal surface of the toothrow; infraorbital canals opening directly above or slightly posterior to the incisive foramina; anterior enamel plate of the first lower premolars recurved, frequently forming a shallow re-entrant angle; anterior enamel plate of the first lower premolars broad and only slightly separated from the posterior enamel plate (rarely continuous), with the lateral enamel plate on the lingual side; diploid chromosome numbers from 40 to 60 (Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024).

Distribution. *Thomomys* ranges from southern British Columbia, Alberta, Saskatchewan, and Manitoba southward through California, Nevada, Arizona, and New Mexico; eastward through North Dakota, South Dakota, Nebraska, and Colorado; and from Washington, Oregon, and California east through Manitoba, eastern North Dakota, and South Dakota, southwestern Nebraska, eastern Colorado, and central New Mexico.

Comments. Thaeler (1980) suggests that the current *T. talpoides* could be split into 10–12 separate species. The genetic data show that *T. talpoides* is a species complex and should be reviewed in detail.

Thomomys clusius Coues, 1875

Wyoming pocket gopher, tuza de Wyoming

1875. *Thomomys clusius* Coues, Proc. Acad. Nat. Sci. Philadelphia 27:138. Type locality "Bridger Pass, 18 mi. SW Rawlins, Carbon Co., Wyoming".

Thomomys idahoensis Merriam, 1901

Idaho pocket gopher, tuza de Idaho

1901. *Thomomys idahoensis* Merriam, Proc. Biol. Soc. Washington, 14:114. Type locality "Brich Creek, Clark Co., Idaho".

1. *T. i. confinis* Davis, 1937. Type locality "Gird Creek, near Hamilton, Ravalli Co., Montana". Known only from Ravalli County, Montana.
2. *T. i. idahoensis* Merriam, 1901. For type locality see above. Range in southeastern Idaho and southwestern Montana.
3. *T. i. pygmaeus* Merriam, 1901. Type locality "Montpelier Creek, 6700 ft., about 10 mi. NE Montpelier, Bear Lake Co., Idaho". Range in southwestern Wyoming and southeastern Idaho.

Thomomys mazama Merriam, 1897

Western pocket gopher, tuza del oeste

1897. *Thomomys mazama* Merriam, Proc. Biol. Soc. Washington, 11:214. Type locality "Anna Creek, 6000 ft., near Crater Lake, Mt. Mazama, Klamath Co., Oregon".

1. *T. m. couchi* Goldman, 1939. Type locality "4 mi. N Shelton, Mason Co., Washington". Known only from Mason County, Washington.
2. *T. m. glacialis* Dalquest and Scheffer, 1942. Type locality "prairie 2 mi. S Roy, Pierce Co., Washington". Known only from Pierce County, Washington.
3. *T. m. helleri* Elliot, 1903. Type locality "Goldbeach, mouth of Rogue River, Curry Co., Oregon". Known only from Curry County, Oregon.
4. *T. m. hesperus* Merriam, 1901. Type locality "Tillamook, Tillamook Co., Oregon". Range in coastal area of northwestern Oregon.
5. *T. m. louiei* Gardner, 1950. Type locality "12 mi, NNE Cathlamet (Crown-Zellerbach's Cathlamet Tree Farm), 2500 ft., Wahkiakum Co., Washington". Known only from Wahkiakum County, Washington.

6. *T. m. mazama* Merriam, 1897. For type locality see above. Range in central-western Oregon and northern California.
7. *T. m. melanops* Merriam, 1899. Type locality "timberline at head Soledue River, Olympic Mts., Clallam Co., Washington". Range in northern Olympia peninsula, Washington.
8. *T. m. nasicus* Merriam, 1897. Type locality "Farewell Bend, Deschutes River, Deschutes Co., Oregon". Known only from central-western Oregon.
9. *T. m. niger* Merriam, 1901. Type locality "Seaton (= Mapleton), near mouth Umpqua River (= head tidewater, Siuslaw River), Lane Co., Oregon". Known only from Benton and Lane Counties, Oregon.
10. *T. m. oregonus* Merriam, 1901. Type locality "Ely, near Oregon City, Willamette Valley, Clackamas Co., Oregon". Known only from northwestern Oregon.
11. *T. m. premaxillaris* Grinnell, 1914. Type locality "2 mi. S South Yolla Bolly Mtn., 7500 ft., Tehama Co., California". Known only from Tehama County, California.
12. *T. m. pugetensis* Dalquest and Scheffer, 1942. Type locality "3 mi. S Olympia, Thurston Co., Washington". Known only from Thurston County, Washington.
13. *T. m. tacomensis* Taylor, 1919. Type locality "6 mi. S Tacoma, Pierce Co., Washington". Known only from Tacoma County, Washington.
14. *T. m. tumuli* Dalquest and Scheffer, 1942. Type locality "7 mi. N Tenino, Thurston Co., Washington". Known only from Thurston County, Washington.
15. *T. m. yelmensis* Merriam, 1899. Type locality "Tenino, Yelm Prairie, Thurston Co., Washington". Known only from Thurston County, Washington.

Thomomys monticola J. A. Allen, 1893

Mountain pocket gopher, tuza de las montañas

1893. *Thomomys monticola* J. A. Allen, Bull. Amer. Mus. Nat. Hist., 5:48. Type locality "Mt. Tallac, 7500 ft., El Dorado Co., California".

Thomomys talpoides (Richardson, 1828)

Northern pocket gopher, tuza del norte

1828. *Cricetus talpoides* Richardson, Zool. Jour., 3:518. Type locality "Fixed at near Fort Carlton (Carlton House), Saskatchewan River, Saskatchewan, Canada".

1858. *Thomomys talpoides*: Baird, Mammals, *in* Repts. Expl. Surv .... , 8(1):403.

1. *T. t. aequalidens* Dalquest, 1942. Type locality "Abel Place, 2200 ft., 6 mi. SSE Dayton, Columbia Co., Washington". Known only from southeastern Oregon.
2. *T. t. agrestis* Merriam, 1908. Type locality "Medano Ranch, San Luis Valley, Colorado". Known only from southern-central Colorado.
3. *T. t. andersoni* Goldman, 1939. Type locality "Medicine Hat, South Saskatchewan River, Alberta". Known only from southwestern Alberta.
4. *T. t. attenuatus* Hall and Montague, 1951. Type locality "3½ mi. W Horse Creek P.O., 7000 ft., Laramie Co., Wyoming". Range in southeastern Wyoming and northeastern Colorado.
5. *T. t. bridgeri* Merriam, 1901. Type locality "Harvey's Ranch, Smith Fork, 6 mi. SW Old Fort Bridger, Uinta Co., Wyoming". Range in southeastern Idaho, western and southwestern California.
6. *T. t. bullatus* Bailey, 1914. Type locality "Powderville, Custer Co., Montana". Range in southern Saskatchewan, eastern Montana, and northeastern Wyoming.
7. *T. t. caryi* Bailey, 1914. Type locality "head Trapper Creek, 9500 ft., Bighorn Mts., Bighorn Co., Wyoming". Range in central-northern Wyoming and southern-central- Montana.
8. *T. t. cheyennensis* Swenk, 1941. Type locality "2 mi. S Dalton, Cheyenne Co., Nebraska". Known only from western Nebraska.
9. *T. t. cognatus* Johnstone, 1955. Type locality "Crowsnest Pass, British Columbia". Known only from southeastern British Columbia.
10. *T. t. columbianus* Bailey, 1914. Type locality "Touchet, Walla Walla Co., Washington". Known only from northern-central Oregon.

11. *T. t. devexus* Hall and Dalquest, 1939. Type locality "1 mi. WSW Neppel, Grant Co., Washington". Known only from central-southeastern Washington.
12. *T. t. douglasii* (Richardson, 1829). Type locality "near mouth Columbia River, probably near Vancouver, Washington". Known only from southwestern Washington.
13. *T. t. durranti* Kelson, 1949. Type locality "Johnson Creek, 14 mi. N Blanding, 7500 ft., San Juan Co., Utah". Range in eastern Idaho and western-central Colorado.
14. *T. t. falcifer* Grinnell, 1926. Type locality "Bells Ranch, 6890 ft., Reese River Valley, Nye Co., Nevada". Known only from central Nevada.
15. *T. t. fisheri* Merriam, 1901. Type locality "Beckwith, Sierra Valley, Plumas Co., California". Range in western California and eastern Nevada.
16. *T. t. fossor* Allen, 1893. Type locality "Florida, 7200 ft., La Plata Co., Colorado". Range in northeastern Arizona, southwestern Colorado, and northern New Mexico.
17. *T. t. fuscus* Merriam, 1891. Type locality "Summit Creek in mountains, head Big Lost River, Custer Co., Idaho". Known only from central Idaho.
18. *T. t. gracilis* Durrant, 1939. Type locality "Pine Canyon, 6600 ft., 17 mi. NW Kelton, Boxelder Co., Utah". Range in northwestern Idaho, northern, central, and northeastern Nevada.
19. *T. t. immunis* Hall and Dalquest, 1939. Type locality "5 mi. S Trout Lake, Klickitat Co., Washington". Known only from central-southern Washington.
20. *T. t. incensus* Goldman, 1939. Type locality "Shuswap, Yale District, British Columbia". Known only from southern British Columbia.
21. *T. t. kaibabensis* Goldman, 1938. Type locality "De Motte Park, 9000 ft., Kaibab Plateau, Coconino Co., Arizona". Known only from northern-central Arizona.
22. *T. t. kelloggi* Goldman, 1939. Type locality "West Boulder Creek, Absaroka Mts., 18 mi. SE Livingston, Park Co., Montana". Known only from Park County, Montana.
23. *T. t. levis* Goldman, 1938. Type locality "Seven Mile. Flat, 10,000 ft., 5 mi. N Fish Lake, Fish Lake Plateau, Sevier Co., Utah". Known only from central Idaho.
24. *T. t. limosus* Merriam, 1901. Type locality "White Salmon, Gorge of the Columbia, Klickitat Co., Washington". Known only from southern Washington.
25. *T. t. loringi* Bailey, 1914. Type locality "South Edmonton, Alberta". Known only from central-western Alberta.
26. *T. t. macrotis* Miller, 1930. Type locality "D' Arey Ranch, 2 mi. N Parker, Douglas Co., Colorado". Known only from Douglas County, Colorado.
27. *T. t. medius* Goldman, 1939. Type locality "Silver King Mine, summit Toad Mtn., 6 mi. S Nelson, West Kootenay District, British Columbia". Known only from southeastern British Columbia.
28. *T. t. meritus* Hall, 1951. Type locality "8 mi. N, 19½ mi. E Savery, 8800 ft., Carbon Co., Wyoming". Known only from central-northern Colorado.
29. *T. t. monoensis* Huey, 1934. Type locality "Dexter Creek Meadow, 6800 ft., at confluence Dexter and Wet creeks, Mono Co., California". Range in eastern California and western Nevada.
30. *T. t. moorei* Goldman, 1938. Type locality "1 mi. S Fairview, 6000 ft., Sanpete Co., Utah". Known only from central Utah.
31. *T. t. nebulosus* Bailey, 1914. Type locality "Jack Boyden's Ranch, 3750 ft., Sand Creek Canyon, 15 mi. NE Sundance, Crook Co., Wyoming". Range in western South Dakota and eastern Wyoming.
32. *T. t. ocius* Merriam, 1901. Type locality "Mountainview, Smiths Fork, 4 mi. (by airline) SE Fort Bridger, Uinta Co., Wyoming". Range in southwestern Wyoming, northwestern Colorado, and northeastern Utah.
33. *T. t. oquirrhensis* Durrant, 1939. Type locality "Settlement Creek, 6500 ft., Oquirrh Mts., Tooele Co., Utah". Known only from central-northern Utah.
34. *T. t. parowanensis* Goldman, 1938. Type locality "Brian Head, 11,000 ft., Parowan Mts., Iron Co., Utah". Known only from southern Utah.
35. *T. t. pierreicolus* Swenk, 1941. Type locality "Wayside, Dawes Co., Nebraska". Known only from western Nebraska.
36. *T. t. pryori* Bailey, 1914. Type locality "head Sage Creek, 6000 ft., Pryor Mts., Carbon Co., Montana". Known only from southern Montana.

37. *T. t. quadratus* Merriam, 1897. Type locality "The Dalles, Wasco Co., Oregon". Known only from southeastern Oregon.
38. *T. t. ravus* Durrant, 1946. Type locality "19 mi. N Vernal, 8000 ft., Uintah Co., Utah". Known only from northeastern Utah.
39. *T. t. relicinus* Goldman, 1939. Type locality "Twin Springs, 20 mi. N Minidoka, Snake River Desert, Minidoka Co., Idaho". Known only from central-southern Idaho.
40. *T. t. retrorsus* Hall, 1951. Type locality "Flagler, Kit Carson Co., Colorado". Known only from eastern Colorado.
41. *T. t. rostralis* Hall and Montague, 1951. Type locality "1 mi. E Laramie, 7164 ft., Albany Co., Wyoming". Range in southern-central Wyoming and central Colorado.
42. *T. t. rufescens* Wied-Neuwied, 1839. Type locality "Minnetaree Village, now Old Fort Clark, about 6 mi. S Stanton, Mercer Co., North Dakota". Range in southwestern Saskatchewan and Manitoba south through South Dakota.
43. *T. t. saturatus* Bailey, 1914. Type locality "Silver, near Saltese, Coeur d'Alene Mts., Missoula Co., Montana". Range in northern Idaho, northwestern Montana, and southeastern British Columbia.
44. *T. t. segregatus* Johnstone, 1955. Type locality "Goat Mtn., E side Kootenay River, near Wynndel, British Columbia". Known only from near Wynndel, southeastern British Columbia.
45. *T. t. shawi* Taylor, 1921. Type locality "Owyhigh Lake, 5100 ft., Mt. Rainier, Pierce Co., Washington". Range in central-southern Washington.
46. *T. t. talpoides* (Richardson, 1828). For type locality see above. Range in western Alberta, central Saskatchewan, central-western Manitoba, and northern-central Montana.
47. *T. t. taylori* Hooper, 1940. Type locality "6 mi. NE summit Mt Taylor, about 8900 ft., near Fernandez summer camp, Valencia Co., New Mexico". Known only from Valencia County, central New Mexico.
48. *T. t. tenellus* Goldman, 1939. Type locality "Whirlwind Peak, 10,500 ft., Absaroka Range, Park Co., Wyoming". Range in northwestern Wyoming and southern Montana.
49. *T. t. trivialis* Goldman, 1939. Type locality "near head Big Timber Creek, 5200 ft., about 15 mi. NW Big Timber, Crazy Mts., Sweetgrass Co., Montana". Known only from central Montana.
50. *T. t. uinta* Merriam, 1901. Type locality "Blacks Fork, 10,000 ft., N base Gilbert Peak, Uinta Mts., Summit Co., Utah". Known only from central-northern Utah.
51. *T. t. wallowa* Hall and Orr, 1933. Type locality "Catherine Creek, 3500 ft., 7 mi. E Telocaset, Union Co., Oregon". Known only from northeastern Oregon.
52. *T. t. wasatchensis* Durrant, 1946. Type locality "Midway, 5500 ft., Wasatch Co., Utah". Known only from northern Utah.
53. *T. t. whitmani* Drake and Booth, 1952. Type locality "Whitman National Monument, 750 ft., 6 mi. W Walla Walla, Walla Walla Co., Washington". Known only from Walla Walla County, eastern Washington.
54. *T. t. yakimensis* Hall and Dalquest, 1939. Type locality "Selah, Yakima Co., Washington". Range in central-southern Washington.

Tribe Geomyini (Clade B)

Sub-clade B1

Genus *Cratogeomys* Merriam, 1895

1895. *Cratogeomys* Merriam, N. Amer. Fauna, 8:150. Type species. *Geomys merriami* (Thomas, 1893).

Content. Seven allopatric species are recognized species of *Cratogeomys* are recognized: *Cratogeomys castanops* (Baird, 1852), *Cratogeomys fulvescens* Merriam, 1895, *Cratogeomys fumosus* (Merriam, 1892), *Cratogeomys goldmani* Merriam, 1895, *Cratogeomys merriami* (Thomas, 1893), *Cratogeomys perotensis* Merriam, 1895, and *Cratogeomys planiceps* (Merriam, 1895).

Etymology. The name *Cratogeomys* is derived from the Greek *krataios,* meaning *"*strong"; *geo,* "earth"; and *mys, "*mouse": strong earth mouse, referring to strong and large specimens within the gophers (Jaeger 1955).

Diagnosis. Dorsal pelage in different colors, mainly shades of brown; fur of underparts paler than in the dorsum; tail short and slightly darker dorsally; body compact and cylindrical; hair silky and long, approximately 8.0 mm; skull large and massive; no ridge on the squamosal but a sagittal crest mainly in adult and old males; interorbital region narrower than the rostrum; zygomatic lateral angle with a plate-shaped expansion. The genus *Cratogeomys* can be differentiated from other species of the family Geomyidae by the following characteristics: rostrum wider than the interorbital region, zygomatic without an anterior angle plate-shaped expansion, and third upper molars not clearly showing two lobes (Merriam 1895; Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024).

Distribution. The species of *Cratogeomys* range from Colorado, Kansas, Oklahoma, New Mexico, and Texas southward throughout the Altiplano Mexicana to the Eje Volcánico Trasversal the Oriental Basin, eastern Puebla, western Veracruz, and western Tlaxcala throughout Jalisco, Colima, and Nayarit (Russell 1968a, Hall 1981; Hafner *et al.* (2004, 2005, 2008). All species of *Cratogeomys* have allopatric distribution.

Comments. Revisions of the species in the genus *Cratogeomys* have been performed by Russell (1968b) and Hafner *et al.* (2004, 2005, 2008). The genus *Cratogeomys* comprises two species groups: *castanops* (*C. castanops, C. fulvescens, C. goldmani, C. merriami,* and *C. perotensis*) and *fumosus* (*C. fumosus* and *C. planiceps;* Hafner *et al.* 2004). The revision of *C. castanops* by Hafner *et al.* (2008) considered that it should be split into two different species, with the boundary between the species located at the Nazas River and Sierra de Parras. *C. castanops* is distributed north of this boundary and *C. goldmani* ranges to the south; all other subspecies should be considered junior synonyms. *C. castanops castanops* includes *C. c. angusticeps, C. c. bullatus, C. c. convexus C. c. dalquesti C. c. hirtus, C. c. parviceps, C. c. perplanus, C. c. pratensis, C. c. simulans, C. c. tamaulipensis, C. c. torridus,* and *C. c. ustulatus* as junior synonyms. The subspecies *C. c. consitus* includes the subspecies *C. c. excelsus, C. c. goldmani* (part of the distribution), *C. c. jucundus, C. c. perexiguus, C. c. sordidulus, C. c. subsimus,* and *C. c. surculus* (part of the distribution). *C. goldmani* is distributed south of the Nazas River and Sierra de Parras, and comprises two subspecies: *C. g. goldmani*, which includes the subspecies of *C. castanops*: *C. c. goldmani* as a junior synonym (part of the distribution), *C. c. rubellus,* and *C. c. surculus* (part of the distribution). The subspecies *C. g. subnubilus* includes the subspecies *C. c. subnubilus, C. c. elibatus, C. c. maculatus, C. c. peridoneus,* and *C. c. planifrons.* The populations previously considered subspecies of *C. merriami* (Russell 1968b), namely *C. m. perotensis* and *C. m. fulvescens*, were elevated to full species (Hafner *et al.* 2005). On the other hand, *C. merriami estor*, *C. m. peraltus*, *C. m. irolonis*, and *C. m. saccharalis* are not considered valid subspecies, the first are junior synonyms of *C. perotensis* and the later of *C. merriami* (Hafner *et al.* 2005). *Cratogeomys fumosus angustirostris, C. f. imparilis* and *C. f. tylorhinus* were considered subspecies of *C. fumosus. C. f. angustirostris* comprises *Pappogeomys zinseri* and *P. tylorhinus brevirostris* as junior synonyms. *C. f. fumosus* includes *P. gymnurus gymnurus, P. g. inclarus, P. g. tellus, P. tylorhinus atratus*, *P. t. zodius, and C. zinseri morulus*; *C. f. tylorhinus* includes *P. neglectus* and *C. t. arvalis* (Hafner *et al.* 2004).

The morphological differences that differentiate *Pappogeomys* from *Cratogeomys* include the tail usually naked and less than one-half of the head-and-body length *vs* tail short and slightly darker dorsally; claws on the forefeet larger *vs* smaller; interorbital region wider *vs* narrower than the rostrum; sagittal crest absent *vs* present; zygomatic without an anterior angle and plate-shaped expansion *vs* lateral angles with a plate-shaped expansion (Merriam 1895; Nelson and Goldman 1934; Russell 1968a; Álvarez-Castañeda 2024). The characteristics that differentiate *Pappogeomys* from *Orthogeomys* include size less than 350 mm *vs* size greater than 350.0 mm; body mass less than 600 g *vs* greater than 800 g); third upper molars clearly with two lobes, outer lingual angle well developed*vs* third upper molars not clearly with two lobes, outer lingual angle not well developed; upper premolars usually with an enamel plate on the back restricted to the lingual region *vs* upper premolars never with an enamel plate in the lingual region (Russell 1968a; Hall 1981; Álvarez-Castañeda 2024). The characteristics listed above are easily observed and useful for separating *Pappogeomys* from *Cratogeomys* and *Ortogeomys*, as many of the authors cited previously have demonstrated.

Cratogeomys castanops (Baird, 1852)

Yellow-faced pocket gopher, tuza de cara amarilla

1852. *Pseudostoma castanops* Baird, *in* Rept. Stan bury' s Expl. Surv. ... Great Salt Lake of Utah... , App. C, p. 313. Type locality "prairie road to Bent's Fort, near present town of Las Animas, Bent Co., Colorado".

1985. *Cratogeomys castanops*: Merriam, N. Amer. Fauna, 8:159.

1. *C. c. castanops* (Baird, 1852). For type locality see above. Range from northern Tamaulipas, Coahuila, and Chihuahua north through Colorado, Kansas, Oklahoma, New Mexico, and Texas.

2. *C. c. consitus* Nelson and Goldman, 1934. Type locality "Gallego, 5500 ft., Chihuahua". Range from northern Tamaulipas, Coahuila, Chihuahua, and Sonora south through the Nazas River and Sierra de Parras, Coahuila, and Durango".

Cratogeomys fulvescens Merriam, 1895

Oriental Basin pocket gopher, tuza de la Cuenca Oriental

1895. *Cratogeomys fulvescens* Merriam, N. Amer. Fauna, 8:161. Type locality "Chalchicomula [= Ciudad Serdán), 8200 ft., Puebla".

Cratogeomys fumosus (Merriam, 1892)

Smoky pocket gopher, tuza del centro de México

1892. *Geomys fumosus* Merriam, Proc. Biol. Soc. Washington, 7:165. Type locality "3 mi. W Colima, 1700 ft., Colima".

1948. *Cratogeomys fumosus*: Hooper, Jour. Mamm., 29:302.

1. *C. f. angustirostris* (Merriam, 1903). Type locality "Cerro Patambán, 10,000 ft., Michoacán". Known only from the southwestern central Mexican Plateau.
2. *C. f. fumosus* (Merriam, 1892). For type locality see above. Patchily distributed on western Michoacán, eastern slopes of the Sierra Madre del Sur in Jalisco and Colima.
3. *C. f. imparilis* (Goldman, 1939). Type locality "Pátzcuaro, Michoacán". Patchily distributed in central Michoacán.
4. *C. f. tylorhinus* (Merriam, 1895). Type locality "Tula, 6800 ft., Hidalgo". Patchily distributed across the southeastern central Mexican Plateau.

Cratogeomys goldmani Merriam, 1895

Goldman`s pocket gopher, tuza del Altiplano

1895. *Cratogeomys castanops goldmani* Merriam, N. Amer. Fauna, 8:160. Type locality "Cañitas, Zacatecas".

1987. *Cratogeomys goldmani*: Lee and Baker, Occ. Pap., Mus. Texas Tech Univ. 114:13.

1. *C. g. goldmani* Merriam, 1895. For type locality see above. Ranges from Nazas River and Sierra de Parras, Coahuila, and Durango south through San Luis Potosí and Zacatecas.
2. *C. g. subnubilus* Nelson and Goldman, 1934. Type locality "Cameros, Coahuila". Range from Monterrey, Nuevo León, along western Tamaulipas, and San Luis Potosí.

Cratogeomys merriami (Thomas, 1893)

Merriam's pocket gopher, tuza del Valle de México

1893. *Geomys merriami* Thomas, Ann. Mag. Nat. Hist., ser. 6, 12:271. Type locality "southern Mexico (Probably the Valley of Mexico according to Merriam, N. Amer. Fauna, 8:152, 1895.)"

1982. *Cratogeomys merriami*: Honeycutt and Williams, Jour. Mamm. 63:212.

Cratogeomys perotensis Merriam, 1895

Perote pocket gopher, tuza de Perote

1895. *Cratogeomys perotensis* Merriam, N. Amer. Fauna, 8:154. Type locality "Cofre de Perote, 9500 ft., Veracruz".

Cratogeomys planiceps (Merriam, 1895)

Toluca Volcano pocket gopher, tuza del Nevado de Toluca

1895. *Platygeomys planiceps* Merriam, N. Amer. Fauna, 8:168. Type locality "N slope Volcán de Toluca, 9000 ft., México".

2004. *Cratogeomys planiceps*: Hafner, Spradling, Light, Hafner, and Demboski. Jour. Mamm 85:1178.

Orthogeomys Merriam, 1895

1895. *Orthogeomys* Merriam, N. Arner. Fauna, 8:172. Type species. *Geomys scalops* (Thomas, 1894).

Content. Only one species of *Orthogeomys* is recognized: *O. grandis* (Thomas, 1893).

Etymology. The name *Orthogeomys* is derived from the Greek *orthos,* meaning "straight"; *geo,* "earth"; and *mys, "*mouse". The name refers to the unusual shape of the skull (Merriam 1895).

Diagnosis.— *Orthogeomys* has the largest members of the family Geomyidae; dorsal pelage reddish cinnamon to brownish; fur of underparts paler; body compact and cylindrical; hair sparse and bristly; skull large and massive; ridge on the squamosal joining the temporal in adult and old males; rostrum narrower than the interorbital region, no interorbital constriction; frontal bone wide and inflated; anterior surface of the upper incisors with one deep groove; last upper molars semi-lobular, only one labial groove; an enamel plate covering the front and re-entrant angle edge of the first upper and lower molariforms; first lower molariforms with an enamel plate and first upper molariforms without a small plate on the lingual side; fourth upper premolars without an enamel plate, although a small plate restricted to the lingual end of the wall rarely present (Russell 1968a; Hall 1981; Álvarez-Castañeda 2024).

Distribution. Patchily distributed along the Pacific coast Colima and Jalisco southward to southwestern Honduras, including Guatemala and El Salvador; altitudinal range from near sea level to at least 2,700 m (Hall 1981; Spradling *et al.* 2016).

Comments. *Orthogeomys ciniculus* was considered as a valid species, but the genetic analyses with nDNA and mtDNA show the absence of reciprocal monophyly. Given this finding, it is considered a junior synonym of *O. grandis*.

Orthogeomys grandis (Thomas, 1893)

Giant pocket gopher, tuza gigante del Pacífico

1893. *Geomys grandis* Thomas, Ann. Mag. Nat. Hist., ser. 6, 12:270. Type locality "Dueñas, Guatemala".

1895. *Orthogeomys grandis,* Merriam, N. Amer. Fauna, 8:175.

1. *O. g. alleni* Nelson and Goldman, 1930. Type locality "near Acapulco, Guerrero, Mexico (altitude 2,000 feet)". Range in southeastern Jalisco coastal plains through central Oaxaca.
2. *O. g. alvarezi* Schaldach, 1966. Type locality "ridge above Lachao (pass above Kilometer 183), on road from Oaxaca City to Puerto Escondido, approximately 40 kms. N. San Gabriel Mixtepec, Municipio de Juquila, Oaxaca, Mexico, altitude approximately 1700 m". Known only from San Gabriel Mixtepec, Oaxaca.
3. *O. g. annexus* Nelson and Goldman, 1933. Type locality "Tuxtla Gutierrez, 2600 ft., Chiapas". Known only from Tuxtla Gutiérrez.
4. *O. g. carbo* Goodwin, 1956. Type locality "Escurano, 2500 ft., Cerro de San Pedro, 20 km W Mixtequilla, Oaxaca [Mexico]". Known only from the central coast of Oaxaca.
5. *O. g. cuniculus* Elliot, 1905. Type locality "Zanatepec, Oaxaca". Known only from type locality.
6. *O. g. engelhardi* Felten, 1957. Type locality "Finca El Carmen (1,319 m), Volcán de San Vicente, [San Vicente Department] El Salvador". Known only from type locality.
7. *O. g. felipensis* Nelson and Goldman, 1930. Type locality "Cerro San Felipe, 10 miles north of Oaxaca, Oaxaca, Mexico (altitude 10,000 feet)". Known only from the Central Valleys of Oaxaca.
8. *O. g. grandis* (Thomas, 1893). Type see above. Known only from the volcano arch of Guatemala.
9. *O. g. guerrerensis* Nelson and Goldman, 1930. Type locality "El Limon, in the valley of the Rio de las Balsas about 20 miles northwest of La Union, Guerrero [Mexico]". Known only from the central lowlands of Guerrero.
10. *O. g. huixtlae* Villa R., 1944. Type locality "Finca Lubeca, 12 km NE Huixtla, 850 m, Chiapas [Mexico]". Known only from southeastern Chiapas.
11. *O. g. latifrons* Merriam, 1895. Type locality "Guatemala (exact locality unknown, but probably lowlands of southern Guatemala)". Known only from Guatemala.
12. *O. g. nelsoni* Merriam, 1895. Type locality "Mt. Zempoaltepec, 8000 ft., Oaxaca". Known only from Sierra Norte of Oaxaca.
13. *O. g. pluto* Lawrence, 1933. Type locality "Cerro Cantoral, north of Tegucigalpa, Honduras". Known only from type locality.
14. *O. g. pygacanthus* Dickey, 1928. Type locality "Cacaguatique, 3500 ft. Department of San Miguel, El Salvador". Known only from western and central El Salvador.
15. *O. g. scalops* (Thomas, 1894). Type locality "Tehuantepec, Oaxaca". Known only from the Isthmus of Tehuantepec, Oaxaca.
16. *O. g. soconuscensis* Villa R., 1949. Type locality "Finca Esperanza, 710 m, 45 km (by road) NW Huixtla, Chiapas [Mexico]". Known only from the coast of Chiapas.
17. *O. g. vulcani* Nelson and Goldman, 1931. Type locality "Volcan Santa Maria, Quezaltenango, Guatemala (altitude 9,000 feet)".

Genus *Pappogeomys* Merriam, 1895

1895. *Pappogeomys* Merriam, N. Amer. Fauna, 8:145. Type species. *Geomys bulleri* (Thomas, 1892).

Content. One species of *Pappogeomys* is recognized: *Pappogeomys bulleri* (Thomas, 1892).

Etymology. The name *Pappogeomys* is derived from the Greek *pappos,* meaning "grandfather"; *geo,* "earth", and *mys,* "mouse": the grandfather of the earth mouse (Jaeger 1955).

Diagnosis. Buller's pocket gopher is medium-sized; total length 200.0 mm to 249.0 mm, skull length 39.2 mm to 42.9 mm. Pelage medium in length and soft, except in coastal populations which have short and sparse pelage; color light brown to dark gray; fur of underparts paler than in the dorsum; most specimens with a small nasal patch consisting of white or pale buffy hairs; body compact and cylindrical; tail usually naked and less than one-half of the head-and-body length; claws on forefeet larger than in *Cratogeomys*; skull large and massive; rostrum wider than the interorbital region; incisors without grooves in the frontal phase; without a zygomatic anterior angle with a plate-shaped expansion (Merriam 1895; Nelson and Goldman 1934; Russell 1968a; (Hafner *et al.* 2009; Álvarez-Castañeda 2024).

Distribution. *Pappogeomys bulleri* is known from the mountains, tablelands, and coastal plains near the western end of the Trans-Mexican Volcanic Belt in west-central México, including the states of Nayarit, Jalisco, and Colima (Hafner *et al.* 2009).

Comments. *Cratogeomys* was first considered a subgenus of *Pappogeomys* (Nelson and Goldman 1934; Russell 1968a); it is currently a full genus (Honeycutt and Williams 1982; Demastes *et al.* 2002). Hafner *et al.* (2009) reviewed the *Pappogeomys bulleri* complex (considering that the previous subspecies of *P. bulleri* are junior synonyms of the following subspecies: *P. b. albinasus* includes *P. b. infuscus* and *P. b. nayaritensis* (part of the species); *P. b. bulleri* includes *P. b. amecensis*, *P. b. flammeus*, *P. b. lagunensis*, and *P. b. lutulentus*; and *P. b. burti* includes *P. b. melanurus. Cratogeomys* differs from all the other species of the family Geomyidae in the following characteristics: rostrum wider than the interorbital region; without a zygomatic anterior angle with a plate-shaped expansion, and third upper molars not clearly showing two lobes.

Pappogeomys bulleri (Thomas, 1892)

Buller's pocket gopher, tuza de Jalisco

1892. *Geomys bulleri* Thomas, Ann. Mag. Nat. Hist., ser. 6, 10:196. Type locality "near Talpa, W slope Sierra de Mascola, 8500 (probably about 5000) ft., Jalisco".

1895. *Pappogeomys bulleri,* Merriam, N. Amer. Fauna, 8:159.

1. *P. b. albinasus* Merriam, 1895. Type locality "Atemajac, a suburb of Guadalajara, Jalisco". Known only from central Jalisco and southeastern Nayarit.
2. *P. b. alcorni* Russell, 1957. Type locality "4 mi. W Mazamitla, 6600 ft., Jalisco". Range in eastern and central Jalisco.
3. *P. b. bulleri* (Thomas, 1892). For type locality see above. Known only from the Sierra Madre del Sur highlands, Jalisco.
4. *P. b. burti* Goldman, 1939. Type locality "Tenacatita Bay, southwestern coast of Jalisco". Known only from coastal areas and lowlands of Colima and Jalisco.
5. *P. b. nayaritensis* Goldman, 1939. Type locality "about 10 mi, S Tepic, 5000 ft., Nayarit". Known only from lowlands of Nayarit.

Zygogeomys Merriam, 1895

1895. *Zygogeomys* Merriam, N. Amer. Fauna, 8:195. Type species *Zygogeomys trichopus* Merriam, 1895.

Content. One species of *Zygogeomys* is recognized: *Zygogeomys trichopus* Merriam, 1895.

Etymology. The name *Zygogeomys* is derived from the Greek *zygos*, meaning "zygomatic"; *geo,* "earth"; and *mys,* "mouse": related to the shape of the zygomatic arch, which is characteristic of the genus (Merriam 1895).

Diagnosis. Body compact and cylindrical; eyes, ears, and limbs small; fore- and hindfoot claws well-developed; tail short and slightly darker dorsally; upper incisors bisulcate, a major sulcus on the inner side of the median line and a minor sulcus on the inner convexity; third upper molars conspicuously bicolumnar, longer than wide owing to the elongation of the posterior loph; rostrum narrow relative to its length; maxillary and squamosal roots of the zygomatic arches in contact above the jugal, and antero-extemal angles rounded rather than expanded; zygomata not widely spreading and slender; sagittal crest short but well-developed (Merriam 1895, Russell 1968a, Álvarez-Castañeda 2024).

Distribution. *Zygogeomys* is known only from Nahuatzen, Pátzcuaro, Cerros Tancítaro, and Patambán, all in the state of Michoacán, Mexico (Merriam 1895, Russell 1968a; Hall 1981).

Comments. *Zygogeomys trichopus* can be found in sympatry with *Cratogeomys fumosus,* which is normally more common and abundant. *Zygogeomys trichopus* can be distinguished from *Cratogeomys* by having two grooves in the anterior surface of the upper incisors (the internal grooves are very notorious, can be detected by passing a pencil tip or a thumb nail).

Zygogeomys can be differentiated from all other genera mainly by having the upper incisors bisulcate, a major sulcus on the inner side of the median line and a minor sulcus on the inner convexity; third upper molars conspicuously bicolumnar, longer than wide owing to the elongation of the posterior loph and maxillary and squamosal roots of the zygomatic arches in contact above the jugalclearly morphologicall distinct from other members of the sub-clade (Merriam 1895, Russell 1968a, Álvarez-Castañeda 2024).

Zygogeomys trichopus Merriam, 1895

Michoacán pocket gopher, tuza de Michoacán

1895. *Zygogeomys trichopus* Merriam, N. Amer. Fauna, 8:196. Type locality "Nahuatzen, Michoacán".

1. *Z. t. tarascensis* Goldman, 1938. Type locality "6 mi. SE Patzcuaro, 8000 ft., Michoacán". Known only from Pátzcuaro area, Michoacán.

2. *Z. t. trichopus* Merriam, 1895. For type locality see above. Known only from Nahuatzen, Cerros Tancítaro, and Patambán, Michoacán.

Sub-clade B2

Genus *Heterogeomys* Merriam, 1895

1895. *Heterogeomys* Merriam, N. Amer. Fauna, 8:179. Type species. *Geomys hispidus* Le Conte, 1852.

Content. Two species of the subgenus *Heterogeomys* are recognized: *Heterogeomys* (*Heterogeomys*) *hispidus* (Le Conte, 1852) and *Heterogeomys* (*Heterogeomys*) *lanius* Elliot, 1905, and five of the subgenus *Macrogeomys* are recognized: *Heterogeomys* (*Macrogeomys*) *cavator* (Bangs, 1902), *Heterogeomys* (*Macrogeomys*) *cherriei* (J. A. Allen, 1893), *Heterogeomys* (*Macrogeomys*) *dariensis* (Goldman, 1912), *Heterogeomys* (*Macrogeomys*) *heterodus* (Peters, 1865), and *Heterogeomys* (*Macrogeomys*) *underwoodi* (Osgood, 1931).

Etymology. The name *Heterogeomys* is derived from the Greek *Hetero,* meaning "other, different"; *geo,* "earth"; and *mys,* "mouse": the other earth mouse or the different earth mouse (Jaeger 1955).

Diagnosis. The genus *Heterogeomys* comprises large specimens; coloration dark brown; pelage harsh and stiff; frontal bone narrow and not markedly inflated; interorbital region decidedly constricted; zygomata more widely spreading; postorbital bar (process) weakly developed; anterior margin of the mesoptergoid fossa even with the plane of the posterior wall of third upper molars; first upper incisors unisulcate; surface of incisors flat on both sides of the sulcus; sulcus wholly on the inner side of the median line and deep and abrupt on the inner third in some specimens; anteroposterior occlusal length of the third upper molars equal to or shorter than the combined lengths of the first and second upper molars; fourth upper premolars with a short enamel plate and restricted to the lingual end of the wall (Russell 1968a; Hall 1981).

Comments. *Heterogeomys* was first described as a full genus (Merriam 1895) and was later considered a subgenus of *Orthogeomys* (Russell 1968a). Molecular data supports its reassignment at the genus level, including *Macrogeomys* as a subgenus (Spradling *et al.* 2016).

Subenus *Heterogeomys* Merriam, 1895

Heterogeomys (*Heterogeomys*) *hispidus* (Le Conte, 1852)

Hispid pocket gopher, tuza gigante tropical

1852. *G*[*eomys*]. *hispidus* Le Conte, Proc. Acad. Nat. Sci. Philadelphia, 6:158. Type locality "near Jalapa, Veracruz".

2016. *Heterogeomys hispidus* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:415

1. *H. h. cayoensis* Burt, 1937. Type locality "Mountain Pine Ridge, 12 mi. S El Cayo, British Honduras [Belize]."
2. *H. h. chiapensis* Nelson and Goldman, 1929. Type locality "Tenejapa, 16 mi. NE San Cristobal, Chiapas". Range in all Chiapas, except northwestern.
3. *H. h. concavus* Nelson and Goldman, 1929. Type locality "Pinal de Amoles, Queretaro". Range in San Luis Potosí, Querétaro, and northwestern Veracruz.
4. *H. h. hispidus* (Le Conte, 1852). For type locality see above. Range in western Veracruz and eastern Puebla.
5. *H. h. hondurensis* Davis 1966. Type locality "8 mi. W Tela, Honduras". Known only from type locality.
6. *H. h. isthmicus* Nelson and Goldman, 1929. Type locality "Jaltipan, Veracruz". Known only from southeastern Veracruz.
7. *H. h. latirostris* Hall and Álvarez, 1961. Type locality "Hda. Tamiahua, Cobo Rojo, Veracruz". Known only from northeastern Veracruz.
8. *H. h. negatus* Goodwin, 1953. Type locality "Gómez Feras [= Farías], 1300 ft., about 45 mi. S Ciudad Victoria, 10 mi. W Pan American Highway, Tamaulipas". Known only from southern Tamaulipas.
9. *H. h. teapensis* Goldman, 1939. Type locality "Teapa, Tabasco. Known only from type locality". Known only from southern Tabasco and northwestern Chiapas.
10. *H. h. tehuantepecus* Goldman, 1939. Type locality "mountains 12 mi. NW Santo Domingo and about 60 mi. N Tehuantepec, 1600 ft., Oaxaca". Known only from northern Oaxaca.
11. *H. h. torridus* Merriam, 1895. Type locality "Chichicaxtle, Veracruz". Known only from central Veracruz.
12. *H. h. yucatanensis* Nelson and Goldman, 1929. Type locality "Campeche, Campeche". Range in Yucatán peninsula, Belize, and Guatemala.

Heterogeomys (*Heterogeomys*) *lanius* Elliot, 1905

Big pocket gopher, tuza gigante de Veracruz

1905. *Heterogeomys lanius* Elliot, Proc. Biol. Soc. Washington, 18:235. Type locality "Xuchil, Veracruz". Known only from type locality.

Subgenus *Macrogeomys* (Peters, 1895)

Heterogeomys (*Macrogeomys*) *cavator* (Bangs, 1902)

Chiriquí Pocket Gopher

1902. *Macrogeomys cavator* Bangs, Bull. Mus. Comp. Zool., 39:42. Type locality "Boquete [Chiriqui Province, Panama] 4,000 to 7,000 feet".

2016. *Heterogeomys cavator* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:416.

1. *H. c. cavator* (Bangs, 1902). For type locality see above. Known only from Chiriqui Province, Panama.
2. *H. c. nigrescens* (Goodwin, 1934). Type locality "El Muñeco (Rio Navarro), 10 miles south of Cartago, Province Cartago, Costa Rica, altitude 4,000 feet". Known only from type locality.
3. *H. c. pansa* (Bangs, 1902). Type locality "Bogaba [Bugaba], Chiriqui Province, Panama". Known only from type locality.

Heterogeomys (*Macrogeomys*) *cherriei* (J. A. Allen, 1893)

Cherrie's Pocket Gopher

1893. *Geomys cherriei* J. A. Allen, Bull. Amer. Mus. Nat. Hist., 5:337. Type locality "Santa Clara, Costa Rica.

2016. *Heterogeomys cherriei* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:416.

1. *H. c. carlosensis* (Goodwin, 1934). Type locality "Cataratos, San Carlos, Province Alajuela, Costa Rica, about 400 feet elevation". Known only from Province Alajuela, Costa Rica.
2. *H. c. cherriei* (J. A. Allen, 1893). For type locality see above. Known only from Santa Clara area, Costa Rica.
3. *H. c. costaricensis* (Merriam, 1895). Type locality "Pacuare, Costa Rica". Known only from Pacuare Basin, Costa Rica.
4. *H. c. matagalpae* (J. A. Allen, 1910). Type locality "Pena [Peña] Blanca, Matagalpa, Nicaragua". Range in central area of Nicaragua.

Heterogeomys (*Macrogeomys*) *dariensis* (Goldman, 1912)

Darién Pocket Gopher

1912. *Macrogeomys dariensis* Goldman, Smiths. Miscl. Coll., 60(2):8. Type locality "Cana (altitude 2,000 feet) in the mountains of eastern Panama".

2016. *Heterogeomys dariensis* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:416.

1. *H. d. dariensis* (Goldman, 1912). For type locality see above. Known only from The Darien, southern Panama.
2. *H. d. thaeleri* (Alberico, 1990). Type locality "ca. 7 km S Bahía Solano, Municipio Bahía Solano, Departamento del Chocó, Colombia, ca. 100 m". Known only from Municipio Bahía Solano northwestern Colombia.

Heterogeomys (*Macrogeomys*) *heterodus* (Peters, 1865)

Variable Pocket Gopher

1865. *Geomys heterodus* Peters, Monatsb. preuss. Akad. Wiss., Berlin, p. 177). Type locality "Costa Rica".

2016. *Heterogeomys heterodus* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:417.

1. *H. h. cartagoensis* (Goodwin, 1934). Type locality "Paso Ancho, Province Cartago, Costa Rica". Central area of Costa Rica.
2. *H. h. dolichocephalus* (Merriam, 1895). Type locality "San Jose, Costa Rica". Known only from the area of San José, Costa Rica.
3. *H. h. heterodus* (Peters, 1865). For type locality see above. Known only from the area of Escazú, Costa Rica.

Heterogeomys (*Macrogeomys*) *underwoodi* (Osgood, 1931)

Underwood's Pocket Gopher

1931. *Macrogeomys underwoodi* Osgood, Field Mus. Nat. Hist., Publ. 295, Zool. Ser., 18(5):143. Type locality "Alto de Jabillo Pirris, between San Geronimo and Pozo Azul, western Costa Rica".

2016. *Heterogeomys underwoodi* Spradling, Demastes, Hafner, Milbach, Cervantes, and Hafner, Jour. Mamm. 97:417.

Sub-clade B3

Genus *Geomys* Rafinesque, 1817

1817. *Geomys* Rafinesque, Amer. Month. Mag., 2:45. Type *Geomys pinetis* Rafinesque, 1817.

Content. Thirteen allopatric species are recognized species of *Geomys* are recognized: *Geomys arenarius* Merriam, 1895, *Geomys attwateri* Merriam, 1895, *Geomys breviceps* Baird, 1855, *Geomys bursarius* (Shaw, 1800), *Geomys jugossicularis* Hooper, 1940, *Geomys knoxjonesi* Baker and Genoways, 1975, *Geomys lutescens* Merriam, 1890, *Geomys mobilensis* (Merriam, 1895), *Geomys personatus* True, 1889, *Geomys pinetis* Rafinesque, 1817, *Geomys streckeri* Davis, 1943, *Geomys texensis* Merriam, 1895, *Geomys tropicalis* Goldman, 1915.

Etymology. The name *Geomys* is derived from the Greek *geo,* "earth", and *mys, "*mouse": earth mouse (Álvarez-Castañeda and Álvarez 1996).

Diagnosis. Dorsal pelage reddish to grayish brown; fur of underparts paler than in the dorsum; back of the rostrum and head darker, contrasting with the nape and back; rump generally paler; whitish gray coloration from chin to neck; body compact and cylindrical; eyes, ears, and limbs small; claws of limbs well-developed; skull large and massive; ridge on the squamosal joining the temporal in adult and old males; interorbital region narrower than the rostrum; middle part of anterior surface of upper incisors with two grooves: one large, deep, and medial; the other small and flanked on the inner side; first upper molars without an enamel plate and larger than the first lower molars; upper molariforms not prominently bicolumnar and almost as long as wide; all molariforms elliptical with a small anterior-posterior axis; anterior and posterior margins of molariforms with enamel, other margins with dentin; sagittal crest poorly developed (Russell 1968a; Baker and Williams 1974; Sudman *et al.* 2006; Álvarez-Castañeda 2024).

Distribution. *Geomys* ranges east of the Mississippi River in Alabama, Georgia, and Florida, associated with deep sandy soils and open areas in long-leaf pinewood forests, and west of the Mississippi River from southern Manitoba, Canada, southward throughout northeastern Tamaulipas, Mexico, and westward throughout eastern Wyoming, Colorado, central New Mexico, western Texas, and northern Chihuahua.

Comments. *G. p. colonus, G. p. cumberlandius,* and *G. p. floridanus* were recognized as distinct species (Hall 1981; Laerm 1981), but not by Patton (1993, 2005) and Baker *et al.* (2003). The subspecies *G. p. austrinus, G. p. colonus, G. p. cumberlandius, G. p. floridanus, G. p. goffi, G. p. mobliensis* are considered junior synonyms of *G. p. pinetis* (Williams and Genoway 1980). Sudman *et al.* (2006) considered that *G. p. mobilensis* may represent a species different from *G. pinetis.* No other species of the genus *Geomys* occurs anywhere near its range. *G. pinetis* cannot be differentiated morphologically from any other species west to the Mississippi River. The chromosome number is 2n = 42, FN = 80.

Geomys shows a high genetic difference (17.6 % [16.1 %–19.0 %]) relative to all other species east of the Mississippi River, higher than the difference within species within any genus of the family Geomyidae and the species east of the Mississippi River 12.4 % (2.8 %–15.3 %). Additiionally, *Geomys* compared to all other species of eastern of the Missiissippi River have presence-absence of lice species (Hafner *et al.* 1994), protein (Kennedy 1988), mtDNA restriction patterns, and allozyme loci (Avise *et al.* 1979), with the Mississippi River acting as an impassable barrier that isolated *Geomys* from all other species of eastern of the Mississippi River.

Geomys shows a high inner variation, so its analysis considered two geographical groups in relation to the Mississippi River determined based on the following conditions: 1) presence of different species complexes determined by several authors (Russell 1968a; Williams and Genoway 1980; Hall 1981; Sudman *et al.* 2006); 2) the marked difference in the nasal shape, being straight in western specimens *vs* nasals with a strong constriction near the middle in eastern specimens (hourglass-shaped**);** interorbital area narrow (western) *vs* a notorious narrow interorbital area (eastern); the east-of-Mississippi population is a different clade from the population thriving in the west side, and is considered basal (Russell 1968a; Penney and Zimmerman 1976; Sudman *et al.* 2006). The Mississippi River is a mayor barrier for the gopher dispersion, flows from northern Minnesota to the Gulf of Mexico, dividing inland plains by a large stream that is virtually impossible to cross by gophers. We consider the crossing of the river as a hypothesis, which a population was located near the boundary of a meander belt and dispersed into the neck of one lobe of the river that later was cut-off from the mainstream, the population could have crossed the river and diversified. The Mississippi River was established as a geographic barrier that prevented subsequent dispersion events.

Geomys arenarius Merriam, 1895

Desert pocket gopher, tuza del desierto

1895. *Geomys arenarius* Merriam, N. Amer. Fauna, 8:139. Type locality "El Paso Co., Texas".

1. *G. a. arenarius* Merriam, 1895. For type locality see above. Range in southern New Mexico, northern Chihuahua, and southwestern Texas.
2. *G. a. brevirostris* Hall, 1932. Type locality "E edge [white] sand [9 mi. W Tularosa], Tularosa-Hot Springs Road, Otero Co., New Mexico". Known only from central-southern New Mexico.

Geomys attwateri Merriam, 1895

Attwater´s pocket gopher, tuza de Corpus Christi

1895. *Geomys breviceps attwateri* Merriam, N. Amer. Fauna, 8:135. Type locality "Rockport, Texas".

1. *G. a. ammophilus* Davis, 1940. Type locality "Cuero, De Witt Co., Texas". Known only from the Brazos River Basin near the coast, central-southeastern Texas.
2. *G. a. attwateri* Merriam, 1895. For type locality see above". Known only from the Colorado and Guadalupe River basins near the coast of southeastern Texas.

Geomys breviceps Baird, 1855

Baird´s pocket gopher, tuza texana del este

1855. *Geomys breviceps* Baird, Proc. Acad. Nat. Sci. Philadelphia, 7:335. Type locality "Prairie Mer Rouge, Lousiana. Known only from Morehouse Parish".

1. *G. b. breviceps* Baird, 1855. For type locality see above. Range from southeastern Oklahoma and southwestern Arkansas south through eastern Texas and western Louisiana.
2. *G. b. ozarkensis* Elrod, Zimmerman, Sudman, and Heidt, 2000. Type locality "from 3 mi S Melbourne, Izard County, Arkansas". Range in northern-central Arkansas.
3. *G. b. sagittalis* Merriam, 1895. Type locality "Clear Creek, Galveston Bay, Galveston Co., Texas". Known only from Galveston Bay, Texas.

Geomys bursarius (Shaw, 1800)

Plains pocket gopher, tuza de las planicies

1800. *Mus bursarius* Shaw, Trans. Linnean Soc. London, 5:227. Type locality "somewhere in upper Mississippi Valley (Restricted to Elk River, Sherburne Co., Minnesota, by Swenk, Missouri Valley Fauna, 1:6.)".

1829. *Geomys bursarius,* Richardson, Fauna BorealiAmericana 1:203.

1. *G. b. bursarius* (Shaw, 1800). For type locality see above. Range from southern Manitoba south through eastern Dakotas, Minnesota, and northwestern Wisconsin.
2. *G. b. illinoensis* Komarek and Spencer, 1931. Type locality "1 mi, S Momence, Kankakee Co., Illinois". Range in central Illinois and central-western Indiana.
3. *G. b. majusculus* Swenk, 1939. Type locality "Lincoln, Nebraska". Range from Iowa and northern-central Missouri west through southeastern Nebraska and eastern Kansas.
4. *G. b. missouriensis* McLaughlin, 1958. Type locality "2 mi. N Manchester, St. Louis Co., Missouri". Range in eastern Missouri.
5. *G. b. wisconsinensis* Jackson, 1957. Type locality "Lone Rock, Richland Co., Wisconsin". Range in western Wisconsin.

Geomys jugossicularis Hooper, 1940

Hall´s pocket gopher, tuza de las planicies centrales

1940. *Geomys lutescens jugossicularis* Hooper, Occas. Pap. Mus. Zool., Univ. Michigan, 420:1. Type locality "Lamar, Prowers Co., Colorado".

1. *G. j. halli* Sudman, Choates and Zimmerman, 1987. 1 3/4 mi. E Ellis (Tl3S, R20W, NE 1/4 Sec. 10), Ellis Co., Kansas. Range from eastern Colorado east through southwestern Kansas, Oklahoma, and northeastern New Mexico.
2. *G. j. jugossicularis* Hooper, 1940. For type locality see above. Oklahoma and southwestern Kansas.

Geomys knoxjonesi Baker and Genoways, 1975

Jones´s pocket gopher, tuza texana del oeste

1975. *Geomys bursarius knoxjonesi* Baker and Genoways, Occas. Pap. Mus. Texas Tech Univ., 29:1. Type locality "4.1 mi. N, 5.1 mi. E Kermit, Winkler Co., Texas".

Geomys lutescens Merriam, 1890

Sandy hill pocket gopher, tuza arenera

1890. *Geomys bursarius lutescens* Merriam, N. Amer. Fauna, 4:51. Type locality "sandhills on Birdwood Creek, Lincoln Co., Nebraska".

1. *G. l. industrius* Villa and Hall, 1947. Type locality "1 1/2 mi. N Fowler, Meade Co., Kansas". Known only from southwestern Kansas.
2. *G. l. lutescens* Merriam, 1890. For type locality see above. Range from South Dakota south through central-eastern Colorado and central Kansas.
3. *G. l. major* Davis, 1940. Type locality "8 mi. W Clarendon, Donley Co., Texas". Range from southern Kansas south through.

Geomys personatus True, 1889

Texas pocket gopher, tuza texana del sur

1889. *Geomys personatus* True, Proc. U.S. Nat. Mus., 11:159 for 1888. Type locality "Padre Island, Cameron Co., Texas".

1. *G. p. davisi* Williams and Genoways, 1981. Type locality "3 mi N, 2.8 mi W Zapata, Zapata Co., Texas". Known only from southern Texas.
2. *G. p. fallax* Merriam, 1895. Type locality "S side Nueces Bay, Nueces Co. Texas". Known only from southeastern Texas.
3. *G. p. fuscus* Davis, 1940. Type locality "Fort Clark [Bracketville], Kinney Co., Texas". Known only from Kinney and Valverde counties, southern Texas.
4. *G. p. maritimus* Davis, 1940. Type locality "Flour Bluff, 11 mi. SE Corpus Christi, Nueces Co., Texas". Known only from Baffin Bay and Flour Bluff, southern Texas.
5. *G. p. megapotamus* Davis, 1940. Type locality "4 mi. SE Oilton, Webb Co., Texas". Range in southern Texas and northeastern Tamaulipas.
6. *G. p. personatus* True, 1889. For type locality see above. Known only from the Mustang and Padre islands, southern Texas.

Geomys pinetis Rafinesque, 1817

Southeastern pocket gopher, tuza del sureste

1806. *Mus tuza* Barton, Mag. fur den neuesten Zustand der Naturkunde (ed. J. H. Voight), 12(6):488 (Type locality restricted to pine barrens near Augusta, Georgia, by Bangs, Proc. Boston Soc. Nat. Hist., 28:175. According to Harper, Proc. Biol. Soc. Washington, 65:36, 952, *tuza* of Barton is of uncertain application and is regarded as not available.)

1817. *Geomys pinetis* Rafinesque, Amer. Month. Mag., 2(1):45. Type locality "Georgia in the region of the pines (More restrictedly, Screven County according to Harper, Proc. Biol. Soc. Washington, 65:36, 1952.)" Regarded as identical with *tuza* by Merriam, N. Amer. Fauna, 8:113, January 31, 1895.

1. *G. p. fontanelus* (Sherman, 1940). Type locality "7 mi. NW Savannah, Chatham Co., Georgia". Known only from Chatham County, eastern Georgia.
2. *G. p. pinetis* (Rafinesque, 1817). For type locality see above. Range in Georgia and Florida.

Geomys mobilensis Merriam, 1895

Southeastern Pocket Gopher of Mobile Bay, tuza del sureste de la bahía de Mobile

1895. *Geomys tuza mobilensis* Merriam, N. Amer. Fauna, 8:119. Type locality "Point Clear, Mobile Bay, Baldwin Co., Alabama".

2006. *Geomys mobilensis*: Sudman, Wickliffe, Horner, Smolen, Bickham, and Bradley, J. Mamm. 87:674.

Geomys streckeri Davis, 1943

Strecker´s pocket gopher, tuza texana del Carrizo

1940. *Geomys personatus minor* Davis, Texas Agric. Exp. Station Bull., 590:29. Type locality "Carrizo Springs, Dimmit Co., Texas". Not *Geomys minor* Gidley, 1922, a fossil. Known only from type locality.

Geomys texensis Merriam, 1895

Llano pocket gopher, tuza del centro de Texas

1895. *Geomys texensis* Merriam, N. Amer. Fauna, 8:137. Type locality "Mason, Mason Co., Texas.

1. *G. t. bakeri* Smolen, Pitts and Bickham, 1993. Type locality "1 mile E D'Hanis, Medina Co., Texas". Known only from Medina, Uvalde, and Zavala counties, central-south Texas.
2. *G. t. llanensis* Bailey, 1905. Type locality "Llano, Texas". Known only from Gillespie, Kimble, and Zavala counties, central-southern Texas.
3. *G. t. texensis* Merriam, 1895. For type locality see above. Known only from Mason, McCulloch, and San Saba counties, central Texas.

Geomys tropicalis Goldman, 1915

Tropical pocket gopher, tuza tropical

1915. *Geomys personatus tropicalis* Goldman, Proc. Biol. Soc. Washington, 28:134. Type locality "Altamira, Tamaulipas".

Supplementary material

Supplementary material 1. GenBank accession numbers for the cytochrome b and cytochrome oxidase subunit 1 sequences and the authors who recorded the sequences in GenBank used in the present study.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6153/1468

Supplementary material 2. Phylogenetic inference using neighbor-joining for the cytochrome b sequences using the large set of data.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6153/1469

Supplementary material 3. Phylogenetic inference using neighbor-joining, unweighted pair group method with arithmetic mean (UPGMA), maximum-parsimony, maximum-likelihood, and Bayesian inference for the cytochrome b and cytochrome oxidase subunit 1 sequences.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6153/1470

Supplementary material 4. Percentage of genetic differences of Cytb between the species examined in the family Geomyidae.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6153/1471

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Hybrid zone between *Ctenomys lami* and *C. minutus*: habitat alterations influence the evolutionary history of two burrowing rodent species of southern Brazil

Thales R. O. de Freitas[1]*, and Simone S. F. Ximenes[1]

[1] Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, CEP 91.501-970, Porto Alegre, Rio Grande do Sul, Brazil. Email: thales.freitas@ufrgs.br (TROF), mone.poa@gmail.com (SSFX).
* Corresponding author: https://orcid.org/0000-0002-1019-9303.

Hybridization events provide insights into central questions in evolutionary biology regarding the role of reproductive isolation in speciation mechanisms. Because of that, there is increasing interest in understanding the patterns and processes underlying hybrid zones. The sister species *Ctenomys minutus* (2n = 48a) and *Ctenomys lami* (2n = 56) are burrowing rodents endemic to the southern Brazilian coastal plain and collectively exhibit high karyotype polymorphism. We analyzed chromosomal and mitochondrial DNA (mtDNA) variation to assess their evolutionary history and confirm and characterize an interspecific hybrid zone between the species. Despite the absence of reciprocal monophyly of mtDNA lineages, *C. minutus* and *C. lami* maintain distinct geographical distributions as well as cytogenetic and morphological differences, suggesting that these incipient species are in the early stages of evolutionary differentiation. The 19 hybrid individuals we include in this analysis show intermediate karyotypic forms between the parental types. These admixed individuals all shared the same mtDNA haplotype that is otherwise only found in *C. minutus*. Although *C. minutus* and *C. lami* are incipient species, their unique differences require distinct conservation efforts. Conservation efforts should focus on maintaining the integrity of pure populations of both species and minimizing anthropogenically induced hybridization.

Los eventos de hibridación brindan información sobre cuestiones centrales en biología evolutiva con respecto al papel del aislamiento reproductivo en los mecanismos de especiación. Debido a eso, existe un creciente interés en comprender los patrones y procesos subyacentes a las zonas híbridas. Las especies hermanas *Ctenomys minutus* (2n = 48a) y *Ctenomys lami* (2n = 56) son roedores excavadores endémicos de la llanura costera del sur de Brasil y colectivamente exhiben un alto polimorfismo cariotípico. Analizamos la variación cromosómica y del ADN mitocondrial (ADNmt) para evaluar su historia evolutiva y confirmar y caracterizar una zona híbrida interespecífica entre las especies. A pesar de la ausencia de monofilia recíproca de linajes de ADNmt, *C. minutus* y *C. lami* mantienen distribuciones geográficas distintas, así como diferencias citogenéticas y morfológicas, lo que sugiere que estas especies incipientes están en las primeras etapas de la diferenciación evolutiva. Los 19 individuos híbridos que incluimos en este análisis muestran formas cariotípicas intermedias entre los tipos parentales. Todos estos individuos mezclados compartían el mismo haplotipo de ADNmt que, de otro modo, solo se encuentra en *C. minutus*. Aunque *C. minutus* y *C. lami* son especies incipientes, sus diferencias únicas requieren esfuerzos de conservación específicos. Los esfuerzos de conservación deben centrarse en mantener la integridad de las poblaciones puras de ambas especies y minimizar la hibridación inducida antropogénicamente.

Keywords: Allopatry; chromosomal polymorphism; cyto-nuclear genome discordance; hybrid zone; incomplete lineage sorting.

Introduction

The concepts of species, species boundaries, and the process of speciation remain the central focus of evolutionary biology. Hybridization may influence the evolutionary process, and its consequences will be determined by the genetic, morphological, chromosomal, and ecological characteristics of the species that come into contact (Barton 2001; Mallet *et al.* 2008). Because of that, hybrid zones are considered laboratories for evolutionary studies, providing insights into the patterns and processes of geographical genetic variation among taxa and the mechanisms of reproductive isolation and speciation (Dowling and Secor 1997).

Hewitt (1988) considered a contact or hybridization zone a region where different forms meet, mate, and produce hybrids. Contact zones can be classified as (a) primary if the populations are parapatric and exhibit a cline in terms of their genetic differences (Hewitt 1988) or (b) secondary if intermittent gene flow occurs between two otherwise allopatric populations (Hewitt 1988). In hybrid zones where hybrids survive beyond the F_1 generation, interbreeding between them and backcrossing with their parental types may lead to a population with a wide variety of recombinant types (Harrison 1993).

The assessment of individuals' karyotypic information can be a helpful starting point for identifying a hybrid zone since diploid numbers and chromosomal rearrangements are commonly variable among species or even at the intraspecific level and may also substantially influence inter-breeding outcomes. The well-documented examples in the literature of hybrid zones among chromosomal races of mammals were reported for the *Sorex araneus* group (Searle and Wójcik 1998) and *Mus musculus domesticus* (Piálek *et al.* 2005), which were used as models for investigations on the role of chromosomal rearrangements in the speciation process.

Among subterranean rodents, the most studied groups regarding hybridization associated with chromosomal variation have been *Spalax, Thomomys,* and *Geomys* (Nevo 1986; Patton 1973; Patton *et al.* 1979, 1984; Baker *et al.* 1989). More recently, studies conducted on intraspecific and interspecific hybrid zones of subterranean rodents from the genus *Ctenomys* (Gava and Freitas 2002; Gava and Freitas 2003; Castilho *et al.* 2012) have provided important insights into the geographical genetic structure among populations with fixed chromosomal differences (Gava and Freitas 2003, Tomasco and Lessa 2007; Fernandez *et al.* 2012; Lopes and Freitas 2012; Lopes *et al.* 2013; Kubiak *et al.* 2020).

Ctenomys minutus and *Ctenomys lami* are sister species of burrowing rodents from the Torquatus group in the genus *Ctenomys* (Freitas 2001; Parada *et al.* 2011; De Santi *et al.* 2024). Both species are endemic to the coastal plain of southern Brazil (Figure 1). The species are characterized by significant Robertsonian chromosomal polymorphisms (Freitas 2006, 2001). *Ctenomys minutus* has a narrow distribution along the first dune line and sand fields near the coast in Santa Catarina (SC) and Rio Grande do Sul (RS) Brazilian states (Figure 1; Lopes *et al.* 2013; Freitas 2021). This species has diploid numbers ranging from 2n = 42 to 50 and autosomal arm numbers (AN) ranging from 68 to 80, comprising 45 karyotypes. The main karyotypes (2n = 42, 46a, 46b, 48a, 48b, 48c, 50a, and 50b) are distributed parapatrically. Between each pair of parapatric karyotypes, with one exception, there is an intra-specific hybrid zone (Freitas 1997; Gava and Freitas 2002, 2003; Freygang *et al.* 2004; Castilho *et al.* 2012; Lopes *et al.* 2013; Freitas 2021).

Ctenomys lami is geographically restricted to an area of 78 x 12 km, corresponding to inland sandy fields of the RS coastal plain (Figure 1; Freitas 2001). This species has five different diploid numbers (2n = 54, 55, 56, 57, 58) and 10 AN (from 74 to 82 and 84), which combined form 26 karyotypes. Four karyotypic blocks, named Blocks A, B, C, and D, were described for this species considering the Robertsonian rearrangements (Freitas 2007). Two intra-specific hybrid zones were reported for this species, one between blocks A x B and another between blocks C x D (Figure 1 inset; Freitas 2007, 2021).

These species are indistinguishable by external morphology. However, *C. lami* was described as a separate species from *C. minutus* due to differences in their karyotypes (mainly regarding the chromosomal forms and diploid numbers), the distinct habitats that the species occupy, and differences in skull morphology (Freitas 2001). The current hypothesis about the process of speciation between *C. minutus* and *C. lami* is based on the role of chromosomal rearrangements and geographical barriers, resulting in an allopatric model of speciation followed by chromosomal differentiation (Freitas 2006).

Cytogenetic data from six individuals sampled in the western margin of Barros Lake, in the state of RS (Figure 1, inset), demonstrated diploid numbers and chromosomal rearrangements intermediate between specimens of *C. lami* (2n = 56) and *C. minutus* (2n = 48a) that inhabit the areas surrounding this region. These individuals have been considered inter-specific hybrid forms between *C. lami* and *C. minutus* (Gava and Freitas 2003). The authors suggested that this hybrid zone is a product of secondary contact. Historically, a wide marsh area west of Barros Lake represented a geographical barrier isolating *C. minutus* and *C. lami*. However, around the 1950s, the introduction of rice cultivation in the region completely drained the marsh, exposing a sandy area, which allowed interspecific contact, mating, and, consequently, the establishment of hybrids. The extent and consequences of that hybrid zone for both species are uncertain and require further investigation.

The goals of the present article are to i) describe the hybrid zone between both species, ii) describe the evolutionary history of *C. lami* and *C. minutus* in the southern Brazilian coastal plain, and iii) provide information to help future conservation decisions, and management strategies for both species. To address these issues, we examine mitochondrial DNA (mtDNA) control region and cytochrome c oxidase subunit I sequences coupled with cytogenetic data for each sampled individual.

Materials and methods

Sampling, species identification and study overview. Sampling covered the entirety of the currently known distribution of *C. lami* and *C. minutus* in the coastal plain of southern Brazil. Both species occur in completely different areas, so initial identification was based on their geographic location of capture. The karyotype determined by Freitas (1997, 2001, and 2007) was examined for each specimen.

A total of 166 individuals of *C. lami* were previously sequenced for mitochondrial DNA (mtDNA), comprising 28 sampling sites (Lopes and Freitas 2012). For *C. minutus,* 244 were sequenced from 30 sites (Lopes *et al.* 2013).

Nineteen new individuals were collected from three distinct sites within the area of secondary contact previously described by Gava and Freitas (2003; Table 1, Figure 1). Trapping was conducted using Oneida-Victor n°0 snap-traps.

Cytogenetics. For the 19 possible hybrids, we determined the diploid and autosomal arm numbers through analyses of at least 20 metaphase spread cells stained with Giemsa following Ford and Hamerton (1956). Meiotic analyses were conducted for males using the technique of verifying the behavior of chromosomes and Robertsonian rearrangements during meiosis (Ford and Evans 1969).

DNA amplification and sequencing. We extracted DNA from the 19 possible hybrids following a modified phenol-chloroform protocol (Sambrook and Russel 2001). Two fragments of mitochondrial DNA (mtDNA) were analyzed. A segment of the HVS1 control region (CR) was amplified using the primers TucoPro (Tomasco and Lessa 2007) and TDKD (Kocher *et al.* 1989). The amplification of cytochrome c oxidase subunit I (COI) followed the protocols suggested by Lopes *et al.* (2013), using the primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994). PCR products were visualized on 1 % agarose gels and purified using Exonuclease I and Shrimp Alkaline Phosphatase (GIBCO-BRL Life Sciences/Invitrogen, Carlsbad, California), following

the guidelines of the suppliers. Sanger sequencing was conducted on an ABI3730 automated sequencer, using the forward primers TucoPro and LCO-1490 for CR and COI, respectively. The electropherograms were visually inspected using Chromas 2.33 (http://www.technelysium.com.au/chromas.html), and ambiguous sequences were reamplified and resequenced. The sequences were aligned using the Clustal W algorithm with default options implemented in Mega 5.2.1(Tamura *et al.* 2011). Alignments were checked and edited by hand.

Genetic variability and genetic differentiation between species and populations. Mitochondrial sequence analyses were performed using CR and COI data sets separately, but most results were achieved using a concatenated data set of CR+COI (CC). Measures of mtDNA genetic diversity, such as the number of polymorphic sites, the mean number of pairwise differences (π), the average number of nucleotide differences (k), the number of haplotypes (H), and haplotype diversity (*Hd*), were calculated using DNAsp 5.10.01 (Librado and Rozas 2009).

Phylogenetic analyses and divergence time estimates. The appropriate model of nucleotide sequence evolution to be applied in the phylogenetic analysis was determined for each mtDNA dataset separately, using the Bayesian Information Criterion (BIC) estimated in jModelTest 2.1.4 (Posada 2008). The HKY + I + Γ and KHY + I provided the best fit for CR and COI data sets, respectively. We conducted Maximum Likelihood (ML) analyses in PhyML 3.0 (Guindon *et al.* 2010). Analyses were seeded with a Neighbor-Joining tree, followed by nearest-neighbor interchange branch-swapping. We evaluated nodal support using 1,000 bootstrap replicates with random taxon addition.

The phylogenetic tree based on a Bayesian Inference (BI), as well the time of the most recent common ancestors (tMRCA) of the main mtDNA clades of *C. lami* and *C. minutus,* were estimated using Beast 1.7.5 (Drummond *et al.* 2012), employing a coalescent prior of constant population size model, under a strict molecular clock, with substitution rates estimated for ctenomyids by Roratto *et al.* (2015). For CR, the rate applied was 2.96×10^{-8} site/year (95 % confidence interval: 1.65×10^{-8} - 4.42×10^{-8}), and for COI, the substitution rate was 2.13×10^{-8} site/year (95 % confidence interval: 1.42×10^{-8} - 2.87×10^{-8}). Both substitution rates and evolution models were applied separately to each partition of the mtDNA data. Analyses were performed following 100 million iterations of MCMC, sampling trees every 10,000 steps and discarding the first 10 % of trees as burn-in. Results were visually inspected using the program Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) and summarized in TreeAnnotator 1.6.1. The BI phylogenetic tree and the inferred tMRCAs for the main clades were recovered using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Trees were outgroup-rooted with homologous sequences of *Ctenomys torquatus*, *Ctenomys pearsoni*, and *Ctenomys ibicuiensi*. Finally, the topological relationships among haplotypes were estimated using Network 4.5.1.0 (http://www.fluxux-engineering.com) with the median-joining approach for the CC data set.

Results

Cytogenetic data. Of the 19 individuals sampled on the western banks of Barros Lake, 18 were successfully karyotyped, 12 of which were hybrid individuals. Comprising six diploid numbers (2n = 48, 50, 53, 54, 55, and 56) and four autosomal arm numbers (AN = 74, 76, 78, and 80), a total of 10 different karyotypes were recovered. The karyotypes for each sampling site are described in Table 2 and in Figure 2. The karyotypic forms of 2n = 56, AN = 80; 2n = 56, AN = 78; and 2n = 55, AN = 76 were previously recorded for *C. lami*, and the form of 2n = 48, AN = 76 was previously described for *C. minutus*. The karyotypes of the putative hybrids were geographically distributed in a gradient through the landscape, ranging from 2n = 56 in individuals from sampling site 1, near populations of *C. lami*. Karyotypes of hybrids were progressively reduced to 2n = 48 in sampling site 3, near populations of *C. minutus* (Figure 1 and Table 2). The sex chromosome pair, with a sub-metacentric X and an acrocentric Y chromosome, was the same for all karyotypes.

In Figure 2, the karyotypes 2n = 56 AN = 80 and AN = 78 belong to the parental forms of *C. lami*. The forms 2n = 55 AN = 76 originated from the crossing of 2n = 54 AN = 80 (backcrossing between 2n = 52 and 2n = 56) and 2n = 56 (*C. lami*). The karyotypes 2n = 54 AN = 80 and AN = 78 originated from the crossing between 2n = 52 and 2n = 56 (*C. lami*). 2n=53 AN=78 and AN=74 originated from the crossing between 2n=52 and 2n=54 (backcrossing 2n=52 x 2n=56). The forms 2n=50 AN=74 and AN=76 originated from the backcrossing between

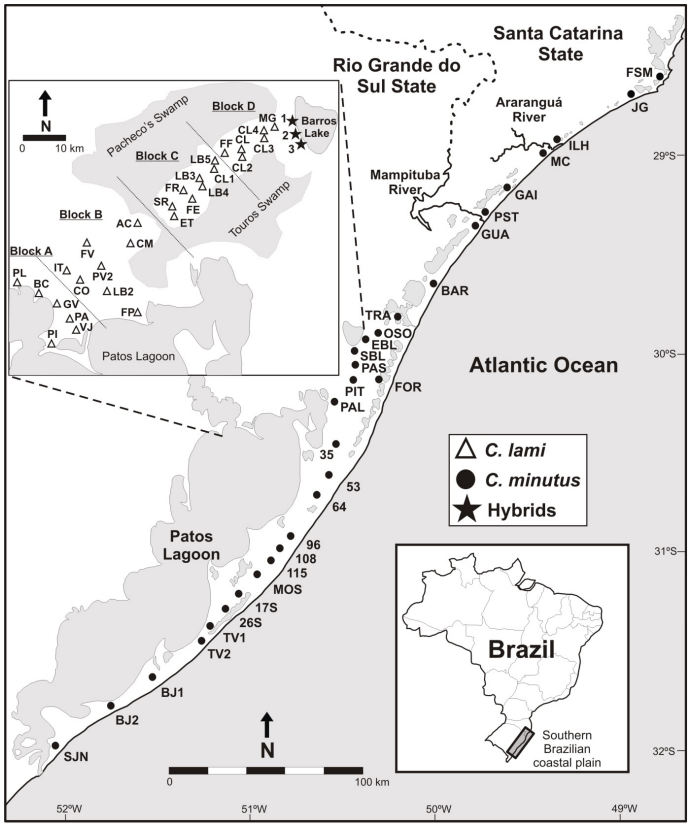

Figure 1. Sampling sites of *C. lami*, *C. minutus*, and interspecific hybrids along the coastal plain of southern Brazil. Locality abbreviations correspond to those in Table 1.

Table 1. Sampling sites, karyotypic and mitochondrial variation observed for *C. lami,* interspecific hybrids, and *C. minutus* and mtDNA clusters. CC haplotype designations follow those shown in the haplotype network (Figure 4).

Locality name	No. S	2n(AN)	CC haplotypes (CR+COI)	mtDNA clusters
Ctenomys lami				
Parque Itapuã (PI)	15	54(76,78)/55a(78,80)	CC55(14)/CC56(1)	BL
Passo da Areia (PA)	3	54(76,78)/55a(76)	CC55(2)/CC57(1)	BL
Varzinha do Jacaré (VJ)	4	54	CC23(4)	BL
Gravatá (GV)	6	56a	CC55(3)/CC23(1)/CC58(2)	BL
Beco do Cego (BC)	11	54(76,82)/55a(76,78,80)/56a(76,78,80)	CC53(10)/CC54(1)	BL
Praia do Lami (PL)	8	-	CC53(8)	BL
Itapuã (IT)	4	58(78,80,83)	CC56(4)	BL
Costa do Oveiro (CO)	9	58(78,79)	CC23(2)/CC54(4)/CC56(1)/CC60(2)	BL
Passo do Vigário 2 (PV2)	2	58(80,82)	CC60(1)/CC61(1)	BL
Fervura (FV)	7	54/58	CC62(7)	BL
Lombas 2 (LB2)	4	57(77)/58(78,80,86)	CC58(3)/CC59(1)	BL
Fazenda Pimenta (FP)	7	58(78)	CC54(2)/CC59(5)	BL
Beco da Macega (BM)	6	58	CC23(2)/CC62(4)	BL
Águas Claras (AC)	3	58(78,80)	CC23(1)/CC59(2)	BL
Estiva (ET)	6	54(74,76,77,78,79)/55a(76)	CC64(4)/CC65(2)	BL
Sanga da Rapadura (SR)	9	54	CC23(3)/CC63(2)/CC64(4)	BL
Fazenda do Estácio (FE)	2	54	CC23(1)/CC66(1)	BL/BC+D
Fazenda Rita Maria (FR)	12	54	CC23(9)/CC66(3)	BL/BC+D
Lombas 4 (LB4)	5	54(75,76,77)	CC23(3)/CC66(2)	BL/BC+D
Lombas 3 (LB3)	6	54(75,76,78)	CC23(1)/CC66(5)	BL/BC+D
Chico Lomã 1 (CL1)	4	54	CC23(1)/CC66(3)	BL/BC+D
Lombas 5 (LB5)	4	54(75,76,78)	CC23(1)/CC66(3)	BL/BC+D
Fazenda dos Freitas (FF)	11	56b(78,80,82)	CC66(7)/CC67(4)	BC+D
Chico Lomã 2 (CL2)	4	-	CC69(4)	BC+D
Chico Lomã (CL)	3	56b(80)	CC66(1)/CC68(2)	BC+D
Chico Lomã 3 (CL3)	6	56b	CC68(6)	BC+D
Chico Lomã 4 (CL4)	-	56b	-	BC+D
Morro Grande (MG)	5	54	CC69(5)	BC+D
1	8	53(74)/54(78)/55(76)/56(78,80)	CC26(8)	BL
2	8	50(74,76)/53(74,80)/54(78,80)	CC26(8)	BL
3	3	48(76)/50(76)	CC26(3)	BL
Ctenomys minutus				
Farol de Santa Marta (FSM)	15	50a(76)	CC48(2)/CC49(8)/CC50(3)/CC51(1)/CC52(1)	N2
Jaguaruna (JG)	17	48c(76)/49a(76)/50a(76)	CC46(14)/CC47(3)	N1/N2
Ilhas (ILH)	15	48c(76)	CC45(15)	N2
Morro dos Conventos (MC)	12	46a(76)	CC43(6)/CC44(6)	N1
Gaivota Beach (GAI)	10	46a(76)	CC42(10)	CO
Passo de Torres (PST)	3	46a(76)	CC39(1)/CC40(1)/CC41(1)	CO
Guarita Beach (GUA)	12	46a(76)	CC38(12)	CO
Barco Beach (BAR)	7	46a(76)	CC35(3)/CC36(1)/CC37(3)	CO

Table 1. Continuation.

Locality name	No. S	2n(AN)	CC haplotypes (CR+COI)	mtDNA clusters
Tramandaí (TRA)	2	46a(76)	CC31(1)/CC34(1)	BL
Osório (OSO)	9	46a(76)	CC25(3)/CC31(2)/CC32(3)/CC33(1)	BL
East Barros Lake (EBL)	5	46a(76)/47a(76)/48a(76)	CC27(5)	BL
South Barros Lake (SBL)	11	47a(76)/48a(76)	CC26(9)/CC28(2)	BL
Passinhos (PAS)	4	48a(76)	CC26(4)	BL
Pitangueira (PIT)	5	48a(76)	CC24(2)/CC25(3)	BL
Fortaleza Lake (FOR)	12	47a(76)/48a(76)	CC26(1)/CC29(1)/CC30(10)	BL
Palmares do Sul (PAL)	7	48a(76)	CC21(5)/CC23(1)/CC24(1)	BL
Road km 35 (35)	4	48a(76)	CC20(3)/CC22(1)	BL/ MO
Road km 53 (53)	5	48a(76)	CC17(2)/CC18(2)/CC19(1)	MO
Road km 64 (64)	3	48a(76)	CC16(2)/CC17(1)	MO
Road km 96 (96)	6	48a(76)	CC13(4)/CC15(2)	MO
Road km 108 (108)	15	42(68,69,70,71,72,73,74)/ 43(70,72,73,74,75)/ 44(72,73,74,75,76)/ 45(74,75,76,78,80)/ 46(71,74,76,77,78)	CC11(15)	MO
Road km 115 (115)	3	42(74)	CC11(2)/CC13(1)	MO
Mostardas (Mos)	16	42(74)	CC11(5)/CC12(1)/CC13(6)/CC14(4)	MO
17 Km south of Mostardas (17S)	10	42(74)/43(74)	CC9(8)/CC10(2)	TA
26 Km south of Mostardas (26S)	15	42(74)/43(70,72,74)/44(74)/46b(76)	CC4(10)/CC8(3)/CC9(2)	TA
Tavares 1 (TV1)	14	46b(76)	CC4(10)/CC5(1)/CC6(3)	TA
Tavares 2 (TV2)	6	46b(76)/47b(76)	CC4(2)/CC6(3)/CC7(1)	TA
Bujuru 1 (BJ1)	12	48b(76,78)	CC3(12)	SO
Bujuru 2 (BJ2)	9	49b(76,77)/50b(76)	CC2(9)	SO
São José do Norte (SJN)	12	50b(76,77)	CC1(12)	SO

No. S – number of samples sequenced; 2n(AN) – diploid and autosomal arm numbers; CC – concatenated mtDNA haplotypes and corresponding number of individuals in parentheses; mtDNA clusters – BL: Barros Lake; BC+D: Blocks C+D; N2: North 2; N1: North 1; CO: Coastal; MO: Mostardas; TA: Tavares; SO: South. Abbreviations of locality names are in parentheses. *Ctenomys lami* localities are in italic font, interspecific hybrids in bold font, and *C. minutus* localities are in normal font.

2n=52 and 2n=48 (*C. minutus*). Cytotype 2n=48 AN=78 is the parental form of *C. minutus*. Pair 1 of *C. minutus* is the largest submetacentric pair of the karyotype in all karyotypes studied in this species (2n=48 AN=76) and is a marker pair for *C. minutus* in this system. It is worth mentioning that this pair does not appear in 2n=56; AN=80 and AN=78 and in 2n=55; AN=76. In karyotypes 2n=54; AN=78, 2n=53; AN=80 and 74 and in 2n=50; AN=76, these two chromosomes appear in pair 1 in the homozygous form. On the other hand, in the forms 2n=54; AN=80 and 2n=50; AN=74 only one chromosome is observed. Two factors reinforce the hybridization between *C. minutus* and *C. lami*: the occurrence of hybrid karyotypes, observed in the 13 animals, different from 2n=48 and 2n=56, and the occurrence of homozygotes for the two submetacentric chromosomes that are the marker pair of *C. minutus*. We obtained cells in spermatogenesis from males collected in the sampling sites 2 and 3. The cells showed meiosis with normal behavior and proper segregation of chromosomes. Two males were analyzed from locality 2, one showed 2n = 50, NA = 74, and gametic cells with n = 25, and another with 2n = 53, AN =

Table 2. Karyotypes of individuals sampled in western banks of Barros Lake.

Sampling sites	No. S	Sex	2n	AN	Bi	Ac
1	2	1 M / 1 F	56*	80	26	28
	2	M	56*	78	24	30
	2	F	55	76	23	30
	1	F	54	78	26	26
	1	F	53	74	23	28
	1	F	54	80	28	24
	1	F	54	78	26	26
2	2	1 M / 1 F	53	80	27	24
	1	F	53	74	23	28
	1	F	50	76	28	20
	1	M	50	74	26	22
3	1	F	50	76	28	20
	2	1 M / 1 F	48±	76	30	16

No.S - number of specimens per karyotype; M – male; F – female; 2n - diploid numbers; AN - autosomal arm numbers; Bi - number of autosomal biarmed chromosomes; Ac - number of autosomal acrocentric chromosomes. *Karytotypic forms previously described for *C. lami*.

± Karytotypic form previously described for *C. minutus*.

Figure 2. Karyotypes obtained in the interspecific hybrid zone between *C. lami* and *C. minutus*.

80, and gametic cells with n = 26 or 27. In locality three only one male was collected with 2n = 48, AN = 76, and gametic cells with n = 24.

Genetic diversity and population differentiation. Mitochondrial DNA sequencing resulted in 398 bp of the control region (CR) and 620 bp of COI, comprising 1,018 bp for CC (CR+COI) data. Measures of genetic variability were higher in *C. minutus* than in *C. lami*, especially those related to the number of nucleotide differences (k and π; Table 3).

Phylogenetic and genealogical relationships among haplotypes. The phylogenetic trees generated by ML and BI analyses showed similar topologies (Figure 3). The main difference was an apparent paraphyly in the ML tree among the clades Coast, Barros Lake, Mostardas, and Block C+D (data not shown). Nonetheless, the low nodal value, even in the BI analysis (gray arrow), confirms the lack of certainty in this portion of the tree.

We found eight well-supported clades based on posterior probabilities (81 – 100) and bootstrap values (63 – 100), six of which exclusively contain haplotypes of *C. minutus* (indicated by a capital M at each tip, Figure 3), one of which exclusively contained haplotypes of *C. lami* (capital L after the tip, Figure 3), and one of which contained haplotypes of both parental species and hybrids (Barros Lake clade, Figure 3). The North 1 (MC and JC) and North 2 (FSM, JG, and ILH) clades correspond to the northern sampling sites of *C. minutus*, and the clades South (BJ2, BJ1, and SJN) and Tavares (26S, 17S, TV1, and TV2),

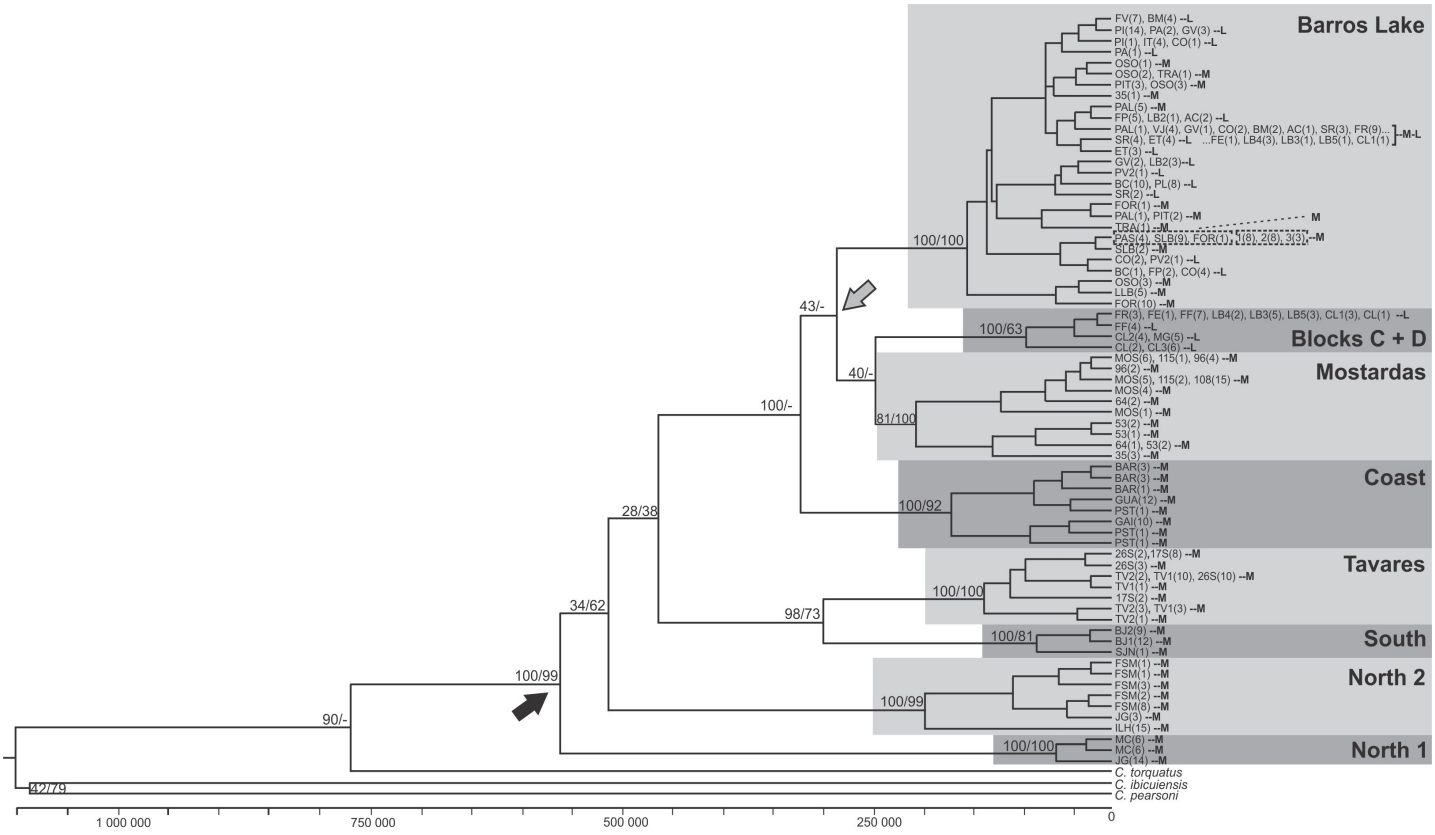

Figure 3. Bayesian phylogenetic tree of mitochondrial DNA concatenated data. Sampling sites are abbreviated according to Table 1 and are followed by the corresponding number of individuals in parentheses as well as a species or hybrid designation: M = *C. minutus*, L = *C. lami*, and H = hybrid. Nodal support is indicated by values above branches for Bayesian Inference and Maximum Likelihood, respectively, - indicates absence of the branch according to each analysis. The eight main mtDNA clades are highlighted by gray squares. Black arrow indicates the most recent common ancestor of all *C. minutus* and *C. lami* individuals, and the grey arrow denotes the node potentially representing the beginning of the divergence process between the two species. Bottom ruler corresponds to the divergence time in years.

represent the southern-most localities of *C. minutus*. The Coast (GAI, PST, GUA, and BAR) and Mostardas (MOS, 115, 96, 108, 64, 53, and 35) clades were also exclusively represented by *C. minutus* specimens. In contrast, the Block C+D clade was comprised only of specimens of *C. lami* sampled in the karyotypic Blocks C (CL1, FR, FE, LB3, LB4, and LB5) and D (FF, CL2, CL3, CL4, CL, and MG, Figure 1).

The Barros Lake clade contains haplotypes common to both species and hybrids. In this group we find all individuals of *C. lami* sampled southeast of the connection between Pachecos and Touros swamps (corresponding to karyotypic Blocks A and B) and some specimens sampled north of this connection (from karyotypic Block C), plus all individuals of *C. minutus* surrounding the Barros Lake region, ranging from sampling sites TRA south to 35, and also the 19 possible hybrids, sampled at the three-starred localities near Barros Lake (Table 1, Figures 1 and 2). The Barros Lake clade showed a star-like shape in the median-joining network (Figure 4), with several low-frequency haplotypes connected by a few mutational steps to a central one (CC23), which is the only haplotype shared between both species. Moreover, all 19 possible hybrids share the same haplotype (CC26), with *C. minutus* specimens sampled from SBL, PAS, and FOR localities.

Divergence time estimates. The estimated divergence time between our in-group and the sister species *C. torquatus* was 762,754 years ago (ya; 95 % Highest Posterior Density (HPD): 413,172 – 1,073,748 ya), and the tMRCA for the in-group was estimated to be 561,666 ya (95 % HPD: 326,433 – 794,706 ya). The divergence times and the 95 % HPD among haplotypes of each of the eight main clades were: North 1: 80,543 ya (8,949 – 112,513 ya); North 2: 206,936 ya (77,502 –301,077 ya); Coast: 182,106 ya (69,535 – 251,475 ya); Barros Lake 166,963 ya (71,030 – 218,147 ya); Blocks C+D: 109,542 ya (17,814 – 159,096 ya); Mostardas: 216,059 ya (96,948 – 301,746 ya); Tavares: 150,457 ya (52,319 – 208,226 ya); and South: 99,300 ya (11,599 – 151,258 ya).

Discussion

Evolutionary history of C. minutus and C. lami in the coastal plain of southern Brazil. Only recently have *Ctenomys minutus* and *C. lami* been considered two separate species due to differences in their karyotypes, areas of occurrence, and skull morphology (Freitas 2001). Our phylogenetic reconstruction based on mtDNA variation shows that the species are not reciprocally monophyletic. Instead, the main clades highlighted the clustering of individuals following a strong pattern of geographic subdivision. This strong geographic association in both species was previously identified in phylogeographical studies (Lopes and Freitas 2012 and Lopes *et al.* 2013). For *C. lami*, mtDNA haplotypes were partially isolated between population blocks

B and C due to the connection between the Pachecos and Touros swamps (Lopes and Freitas 2012). For *C. minutus*, the main subdivisions of mtDNA haplogroups and population clusters were associated with geographic discontinuities throughout the landscape, represented by rivers, paleochannels, and the transition between sandy fields and dunes (Lopes *et al.* 2013, Freitas 2021).

The species from the genus *Ctenomys* are commonly characterized by low rates of adult dispersal among relatively small and fragmented populations, which promote the establishment of small genetic units where genetic variation is low and interpopulation divergence is high (Reig *et al.* 1990; Lessa and Cook 1998; Wlasiuk *et al.* 2003; Gaspareto *et al.* 2024). Considering that the mtDNA has a non-recombinant maternal inheritance and that females of ctenomyids commonly show low rates of dispersal (Malizia and Busch 1991; Cutrera *et al.* 2006), it is expected that the power of taxonomic resolution of the mtDNA is limited when the process of speciation is a recent event, accounting for a pattern of incomplete lineage sorting, in which the gene genealogy may differ from the species phylogeny (Pagès *et al.* 2013).

The estimation of the tMRCA among all individuals of *C. minutus* and *C. lami* was around 562 thousand years ago (kya; black arrow in Figure 3). However, this node age does not necessarily match the starting point of the process of speciation between these species. While the basal relationships among the in-group clades are uncertain, it is possible that the divergence process between *C. minutus* and *C. lami* began at approximately 292 kya, which corresponds to the age estimation of the node that includes both species and hybrids, represented by the clades of Barros Lake, Blocks C+D, and Mostardas (grey arrow in Figure 3). Nonetheless, distinguishing gene tree divergence from population divergence at this stage of species differentiation remains difficult.

Another well-studied complex of species in the genus *Ctenomys* is the Perrensis group, which, like *C. minutus* and *C. lami*, shows discordance in species delimitations across different datasets. Traditionally, based on geographic range, morphology, and chromosomal variation, the Perrensis group is described as formed by three species (*Ctenomys roigi*, *Ctenomys perrensi,* and *Ctenomys dorbignyi*) and several forms of uncertain taxonomic status (*Ctenomys* sp.; Ortells 1995; Caraballo *et al.* 2012; Caraballo and Rossi 2017). Later studies based on chromosomes and mtDNA cytochrome b sequences suggested the existence of two other species in addition to the first three, despite the absence of reciprocal monophyly among clades (Giménez *et al.* 2002). More recently, Fernández *et al.* (2012) analyzed microsatellite and mitochondrial data, which revealed an even more complex evolutionary scenario than previously described. The authors suggested that populations were not sufficiently isolated to complete speciation, and thus, when they come into contact and hybridization takes place, even between populations with different chromosomal numbers, this leads to incongruence among character sets. A similar result was found by Patton and Smith (1994) between *Thomomys bottae* and *T. townsendii,* wherein incongruence among character sets reflected the known complexity of the divergence process, especially in the presence of hybridization. For *C. minutus* and *C. lami*, despite the absence of mtDNA monophyletic clades separating both species and the presence of a hybrid zone, *C. minutus* and *C. lami* maintain distinct geographic distributions as well as cytogenetic and morphological differences, suggesting that these incipient species are at the early stages of the differentiation process.

Table 3. Genetic diversity for *C. lami*, *C. minutus* and interspecific hybrids using the mtDNA concatenated data.

Identification	*N*	No.H	Pol. Sites	*k*	*π*	*Hd*
C. lami	166	18	21	4.63	0.00456 (±0.00021)	0.908 (±0.009)
C. minutus	276	52	87	15.59	0.01533 (±0.00025)	0.965 (±0.003)
Hybrids	19	1	0	0.00	0.00000 (±0.00000)	0.000 (±0.000)

N - number of samples; No.H - number of haplotypes; Pol. Sites - polymorphic sites; k - average nucleotide differences; π - nucleotide diversity; *Hd* - haplotype divergence.

The hybrid zone. The results of mtDNA and cytogenetic data presented here strongly support the hypothesis of a region of hybridization around the western banks of Barros Lake, in RS state, between *C. minutus* and *C. lami* (Figure 1).

In this work, of the 19 individuals sampled in the hybrid zone region, 13 presented chromosomal rearrangements and intermediate diploid numbers between the karyotypic forms of the parental types involved (*C. lami* 2n = 56b, and *C. minutus* 2n = 48a; see Table 2, and Figure 1). Gava and Freitas (2003) found hybrids for the first time in five animals and in another population 5 km further north with 2n = 50 FN = 78, 2n-51 FN = 77, FN =7 8 and FN=80 and 2n = 52 FN =7 9. The wide range of karyotypic forms found among hybrids may be derived from several crossings beyond the F1 generation, as well as backcrossing with parental forms. The supposed low effectiveness of chromosomal differences as barriers to gene flow may be caused by a neutral or weak under-dominant condition of the chromosomal rearrangements, and thus, they may not substantially reduce hybrid fitness (Rieseberg 2001). The heterozygous carriers of chromosomal rearrangements may maintain fertility by means of mechanisms that suppress partial or total recombination with harmful effects during meiosis (Rieseberg 2001).

Regarding mtDNA data, all 19 hybrids shared the same haplotype (CC26; see Figure 3) with *C. minutus* from SBL, PAS, and FOR sampling sites located near the region of hybridization (Figure 1). Since the mitochondrial genome is only maternally inherited, the pattern of mtDNA recovered in hybrids provides evidence that *C. minutus* females are the main, if not the only, donors of the mitochondrial genome to hybrids (Figure 4).

The pattern observed in the hybrid zone described here, in which hybrids carry the mtDNA of *C. minutus,* may have been reached by means of pre and/or post-zygotic isolation

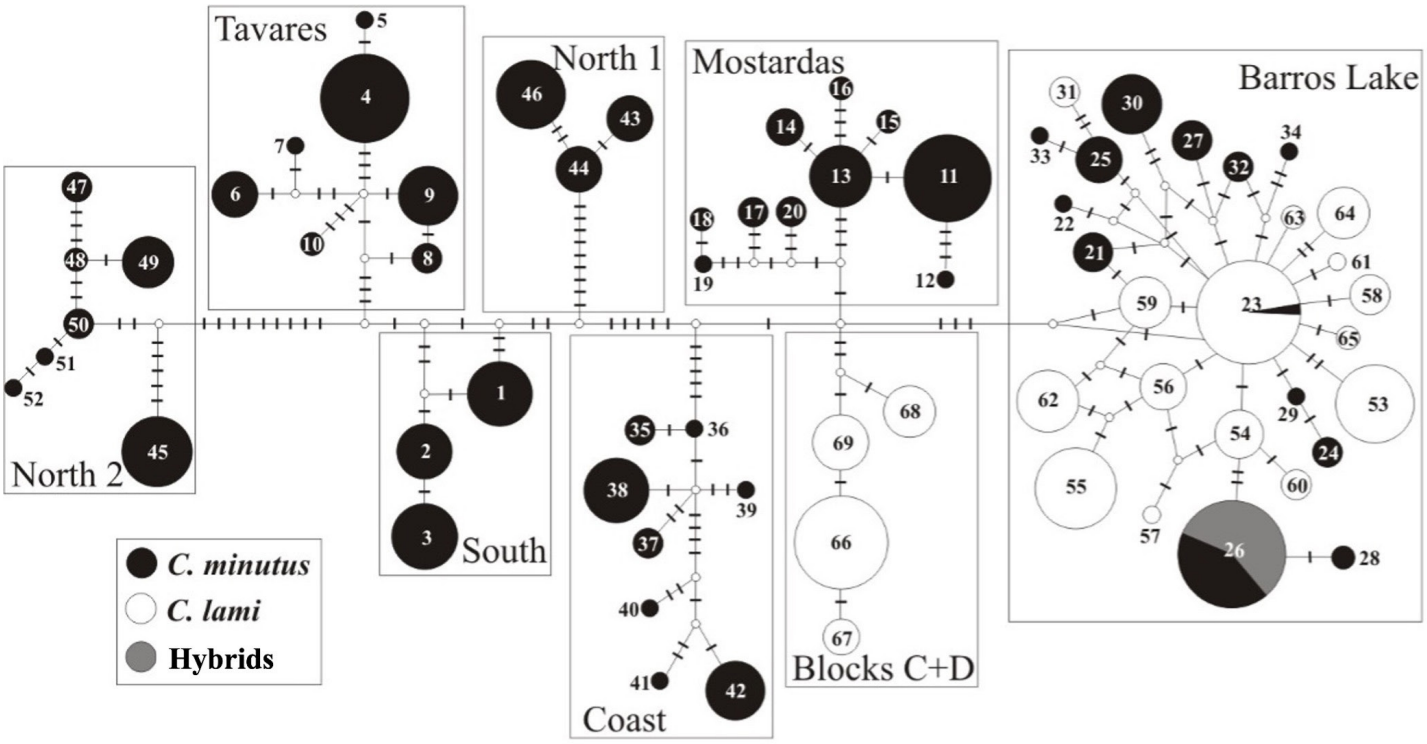

Figure 4. Median-joining haplotype network of mitochondrial DNA concatenated data. The colors represent the species and hybrids following the legend. The area of the circle is proportional to the haplotype frequency, each bar represents one mutational step, and small white dots indicate unsampled haplotypes. Haplotype numbers correspond to those in Table 1. The eight main haplogroups are highlighted by squares.

mechanisms, *e. g*., a mate choice system involving females of *C. minutus* with males of *C. lami*. Sexual selection is an important force in reproductive isolation and speciation, and mate preference represents one of the aspects of this force. Mate choice can be influenced by a series of biological characters (Shurtliff 2013), and female preferences have been described in subterranean rodent hybrid zones in the genus *Thomomys* (Patton and Smith 1993) and *Geomys* (Bradley *et al.* 1991). However, it is difficult to distinguish whether the pattern observed here is due to mate choice and/or the consequence of hybrid inviability/sterility from the mating between females of *C. lami* and males of *C. minutus*. Despite our reduced sample size of hybrids and the fact that we were not able to recover cells in spermatogenesis for males from sampling site 1 due to technical problems, the hybrid males collected in sampling sites 2 and 3 presented meiosis with normal behavior and proper segregation of chromosomes, demonstrating that at least some hybrid males seem to be fertile.

Chromosomal rearrangements mainly involve chromosome inversions, fissions, and centromeric fusions between chromosomes. Such rearrangements are mainly observed in *C. lami* (Freitas 2007). Therefore, chromosomal alterations can cause reproductive incompatibility between different individuals (Ferree and Barbash 2009; Nishino *et al.* 2019; Ono and Greig 2020). The occurrence of only one type of crossing may be related to the fact described by Nishino *et al.* (2019) in two subspecies, *Mus musculus domesticus* and *M. m. molossinus*. Between the crossing of *M. m. domesticus* females and *M. m. molossinus* males, the F1 males are sterile, but F1 males are fertile in the reciprocal parental cross. This is because, in sterile males, apoptosis in spermatocytes is considerably higher than in the parental lines and in the fertile hybrids from the opposite parental cross. Further, analysis of metaphase I in this system showed that some chromosomes do not align in the equatorial plane of the cell, indicating the formation of non-viable gametes. Finally, another cause of hybrid sterility in this system is the non-pairing of the X and Y sex chromosomes and, consequently, the lack of formation of the synaptonemal complex during prophase I (Nishino *et al.* 2019).

In *Ctenomys*, a few hybrid zones between different species have been described. Hybrids are known between *C. flamarioni* (2n = 48) and *C. minutus* (2n = 46a). The crosses of these species have a distinct pattern from that described in this work, as they occur in both parental directions. However, unlike what we find between *C. minutus* and *C. lami*, no karyotypes originated from backcrossing between the F1 and either of the parents (Kubiak *et al.* 2020).

De Queiroz (1998) introduced the concept of species as lineages of independent metapopulations that evolve over time. Thus, characteristics that are useful in diagnosing species, such as distinct phenotypic characteristics, ecological differences, and reciprocal monophyly, arise in different ways across the tree of life due to differences in the pace of evolution of morphological, ecological, behavioral, and genetic factors that impact reproductive isolation (de Queiroz 2007). Our analysis of *C. minutus* and *C. lami* shows that the two species are still differentiating and that they are best characterized as incipient species. Thus, conservation efforts should focus mainly on preserving the integrity of pure populations of both species. Furthermore, although *C. minutus* and *C. lami* are incipi-

ent species, conservation strategies must be considered separately, respecting their particularities.

Implications for conservation. Hybridization and introgression are not usually listed among the main threats to extinction. If the hybridization process occurs naturally, it is considered part of the evolutionary history of the taxa involved. However, these phenomena can become problematic when they result from human actions (Rhymer and Simberloff 1996). Unnatural hybrid zones created by human interventions deserve special attention from conservationists, as they can compromise the genetic integrity and the evolutionary process of the species involved (Rhymer and Simberloff 1996; Allendorf *et al.* 2001).

Hybridization between *C. minutus* and *C. lami* probably originated approximately 70 years ago through human-mediated habitat alteration associated with the introduction of rice cultivation on the western shores of Lake Barros, which dried up a marsh and allowed contact between both species (Gava and Freitas 2003). Considering that hybridization between these species has become quite advanced, with fertile hybrids even mating with each other or with their parental types, it is a difficult process to stop since the existence of hybrid swarms hinders conservation and the recovery of threatened taxa (Allendorf *et al.* 2001).

Ctenomys lami was recently included in the IUCN Red List of Threatened Species as "vulnerable". Studies have shown that the vulnerability of this species is greater than previously assumed, and its extinction could lead to a major loss of genetic diversity since it represents a unique pool of chromosomal variation among *Ctenomys* species. The results obtained by Lopes and Freitas (2012) supported the designation of an ESU and a MU within the geographic area of *C. lami*, which should be considered in developing conservation strategies and implementing protected areas.

Ctenomys minutus is listed in the IUCN Red List of Threatened Species as data deficient. The data provided in this study and Lopes *et al.* (2013) support that, despite having a larger geographic distribution and higher levels of genetic variability than *C. lami, C. minutus* also deserves attention in conservation efforts. In addition to the consequences that hybridization with *C. lami* and *C. flamarioni* (Kubiak *et al.* 2020) may pose to *C. minutus* populations, these species are subject to threats regarding the vulnerability of the coastal plain of southern Brazil, such as global warming and sea level rise, urbanization, coastal armoring, sand mining, construction of jetties, introduction of domestic animals and exotic vegetation (Fernandes *et al.* 2007).

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Appendix I

Data accessibility

Mitochondrial sequence data are deposited at Genbank under the following accession numbers: *Ctenomys minutus* CR (HM236969 to HM237008), and COI (HM237009 to HM237043); *Ctenomys lami* CR (JQ322885 to JQ322898), and COI (JQ322899 to JQ322907); hybrids CR (HM236991), and COI (HM237016); *Ctenomys torquatus* CR (HM443438), and COI (HM443439); *Ctenomys pearsoni* CR (JQ341031), and COI (JQ341042); and, *Ctenomys ibicuiensi* CR (JQ389108), and COI (JQ389074).

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The woodrats of California: evolution across a dynamic landscape

Marjorie D. Matocq[1*], Matthew L. Holding[2], Alexandra Coconis[1], Jim Henderson[3], W. Brian Simison[3], and Kathryn M. Everson[4]

[1] Department of Natural Resources and Environmental Science; Program in Ecology, Evolution and Conservation Biology, University of Nevada, Reno. Reno. Nevada 89509, USA. Email: acoconis@unr.edu (AC), mmatocq@unr.edu (MDM).
[2] Life Sciences Institute, University of Michigan. Ann Arbor. Michigan, USA. Email: venomatt@umich.edu (MLH).
[3] California Academy of Sciences. San Francisco. California, USA. Email:jhenderson@calacademy.org (JH), bsimison@calacademy.org (WBS).
[4] Integrative Biology Department, Oregon State University. Corvallis. Oregon, USA. Email: eversonk@oregonstate.edu (KME).
*Corresponding author: https://orcid.org/0000-0003-4829-3749.

The dynamic landscapes of California have supported the evolution of high levels of biological diversity, including in the genus *Neotoma* (woodrats). Here, we use whole mitochondrial genomes and low coverage genome-wide data to explore patterns of diversity within and among five western lineages of woodrats: *Neotoma fuscipes*, *N. macrotis*, *N. bryanti*, *N. lepida* and *N. cinerea*. We place these patterns of diversity and differentiation within the context of what has been learned about the evolutionary dynamics of these species over the past 25 years. We end by exploring how new genomic datasets coupled with intensive fieldwork will continue to provide new insights into the evolutionary history and future trajectories of these lineages.

Los paisajes dinámicos de California han permitido la evolución de altos niveles de diversidad biológica, incluso en el género *Neotoma* (ratas de bosque). En este artículo, utilizamos genomas mitocondriales completos y datos de baja cobertura de todo el genoma para explorar patrones de diversidad dentro y entre cinco linajes occidentales de ratas de bosque: *Neotoma fuscipes*, *N. macrotis*, *N. bryanti*, *N. lepida* y *N. cinerea*. Ubicamos estos patrones de diversidad y diferenciación en el contexto de lo que se ha aprendido sobre la dinámica evolutiva de estas especies en los últimos 25 años. Terminamos explorando cómo los nuevos conjuntos de datos genómicos, junto con un trabajo de campo intensivo, seguirán brindando nuevos conocimientos sobre la historia evolutiva y las trayectorias futuras de estos linajes.

Keywords: California Floristic Province; hybridization; low coverage genomes; mitogenomes; *Neotoma*.

Introduction

The landscapes of western North America have supported the evolution of diverse biotic communities. In particular, the California Floristic Province is a global biodiversity hotspot (Myers *et al.* 2000) and the state of California has the highest level of endemism and highest number of mammal species in the U.S. (Stein 2002). The topographic diversity of the region has created a landscape characterized by diverse climatic conditions and steep environmental gradients that have led to the diversification of both plant and animal species (Davis *et al.* 2008; Badgley *et al.* 2017).

The woodrats (genus *Neotoma*) of California are among many lineages that have diversified across this landscape (Goldman 1910; Hooper 1938; Matocq 2002a; Patton *et al.* 2007; Hornsby and Matocq 2012). Their evolutionary history provides insight into the timing and magnitude of processes that may have similarly influenced the evolution of many other taxa across western landscapes. Of the ~22 species of woodrats, five lineages are geographically well-represented in California: *Neotoma fuscipes*, *N. macrotis*, *N. lepida*, *N. bryanti*, and *N. cinerea*. The geographic distribution of standing genetic variation in these lineages is the result of multiple evolutionary processes operating at different spatial and temporal scales. That is, historic climate oscillations and barriers to movement determined major patterns of divergence within and among these lineages and set the stage of available regional genetic variation that we see today (Matocq 2002a; Patton *et al.* 2007; Hornsby and Matocq 2012). At a regional scale, current patterns of genetic variation are influenced by environmental gradients in climatic conditions and resource availability (Matocq and Murphy 2007; Shurtliff *et al.* 2013; Dearing *et al.* 2022; Nielsen *et al.* 2023), to which populations are continuously responding through a combination of genetic drift, phenotypic plasticity, and adaptive evolution.

Here, we begin gaining new insight into the various spatial and temporal scales of evolution in these woodrat lineages through the lens of the first whole genome datasets available for these western lineages. We generated whole mitochondrial genome sequences and low coverage resequencing of the nuclear genome from a set of populations representing each of five western lineages of woodrats. We assess support for previous work in these systems and focus on the general hierarchical distribution of genetic variation within and among these lineages, while recognizing that further insight awaits additional geographic and genomic sampling. Our initial questions include: 1) What is the timing of major lineage diversification of the western woodrat lineages?; 2) How do levels of diversity and differentiation compare between mitochondrial and genome-wide nuclear datasets?; and 3) Do we detect patterns of introgression among the western lineages? In addressing

these questions, we also review what has been learned in the past ~25 years about the processes that have generated and maintained genetic variation in these lineages. We propose how genome-scale sequencing data will contribute new insights into the evolutionary history and future trajectories of these lineages.

Background: the woodrats of California. The five western species of *Neotoma* that we include in this analysis comprise the sister species *Neotoma fuscipes* and *N. macrotis*, and *N. lepida* and *N. bryanti* that are members of sister clades, as well as *N. cinerea* that may be sister to the *N. fuscipes/N. macrotis* clade (Matocq *et al.* 2007; Bradley *et al.* 2022), although the long branch leading to *N. cinerea* has made the placement of this taxon problematic (Matocq *et al.* 2007; Steppan and Schenk 2017).

Neotoma fuscipes and *N. macrotis.* *Neotoma fuscipes* (Dusky-footed woodrat) and *N. macrotis* (Big-eared woodrat) are sister lineages that diverged from one another approximately two million years ago (mya; Matocq 2002a). *Neotoma macrotis* occupies oak and riparian woodlands with well-developed shrub understory (Linsdale and Tevis 1951; Matocq 2002a). Their range extends from Monterey Bay on the central coast of California, south along the Coast Ranges to the Sierra de San Pedro Mártir of northern Baja California, across the South Coast, Peninsular, and Transverse Ranges, north along the western foothills of the Sierra Nevada and along the eastern side of the Sierra Nevada to Bishop, California (Figure 1). Their northeastern range limit occurs near the South Fork of the American River (Matocq and Murphy 2007). One isolated population is known in the Granite Mountains of the Mojave Desert, likely a Pleistocene relict from when the species was more widespread across what is currently the Mojave Desert (Smith *et al.* 2000).

Neotoma fuscipes primarily occupies oak, mixed-coniferous, and juniper woodlands (Carraway and Verts 1991; Matocq 2002a). The species ranges from the Columbia River in western Oregon, south throughout much of northern California, extending along the north coast to the San Francisco Bay Area and in the foothills of the Sierra Nevada to the South Fork of the American River (Matocq and Murphy 2007). A distinct genetic lineage of *N. fuscipes* that Matocq (2002a) referred to as the "west central" clade extends from the San Francisco Bay Area south to the Monterey Bay and throughout the inner Coast Ranges (Boria *et al.* 2021). Relatively shallow mtDNA divergences within the northern clade of *N. fuscipes* suggest that this taxon may have only recently (re)-expanded into northern California (Matocq 2002a), yet two regionally distinct genetic groups exist across this portion of their range (Boria *et al.* 2021).

The ranges of *N. macrotis* and *N. fuscipes* are largely parapatric (Matocq 2002a; 2002b), but the two species hybridize when they come into contact. There is evidence of historic hybridization between the two species (Matocq *et al.* 2012) in a now-isolated population of woodrats in the Great Central Valley of California at Caswell Memorial State Park (see Cypher *et al.*, this volume). Patterns of morphological character displacement in the foothills of the Sierra Nevada suggest historic contact between *N. fuscipes* and *N. macrotis* in this region (Matocq and Murphy 2007), but an active area of contact has yet to be discovered in the Sierra Nevada. A zone of secondary contact and active hybridization between the two species exists south of Monterey Bay, California along the Nacimiento River on the Camp Roberts Military Reservation (Coyner *et al.* 2015; Hunter *et al.* 2017; Matocq *et al.* 2024).

Neotoma lepida and *N. bryanti.* *Neotoma lepida* (Desert woodrat) and *N. bryanti* (Bryant's woodrat) are part of two sister clades that diverged from one another approximately 1.6 mya (Patton *et al.* 2007). *Neotoma lepida* primarily occupies desert shrubland habitats, including those dominated by creosote bush and yucca in the Mojave Desert to Juniper and Sagebrush-dominated habitats in the Great Basin Desert (Figure 1). The species is found throughout the lower elevations of the Mojave and Great Basin Deserts and is often replaced by *N. cinerea* at mid and higher elevations throughout the Great Basin (Coconis *et al.* 2024).

Neotoma bryanti occupies similar arid habitats to *N. lepida* in the eastern and southern portions of its range, and coast scrub habitats in the western portion of its range.

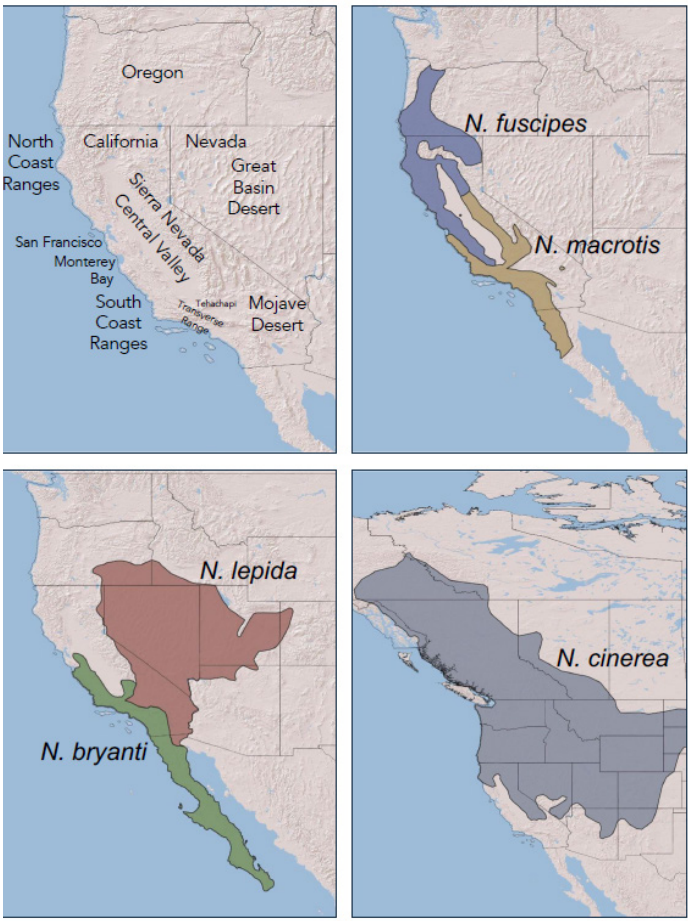

Figure 1. Geographic distribution of five species of woodrats that occur in California and the major topographic features that have determined the biogeographic history of biotic diversity in California.

Both species utilize rock and boulder structures when available, but both can also build free-standing houses. *Neotoma bryanti* ranges from the San Francisco Bay south along the coastal and inner Coast Ranges through northern Baja California, across the Transverse ranges and into the southern foothills of the Sierra Nevada (Figure 1).

The ranges of *N. lepida* and *N. bryanti* are parapatric with two known areas of active hybridization (Patton *et al.* 2007): one in the Kelso Valley of the western Mojave Desert (Shurtliff *et al.* 2014; Jahner *et al.* 2021; Nielsen *et al.* 2023) and the other in the Morongo Valley of southern California (Klure *et al.* 2023). Historic hybridization between the two species has led to a pattern of mitonuclear discordance wherein nuclear and morphological *N. bryanti* of the southern Sierra Nevada, Tehachapi Mountains, and western Mojave Desert possess a *N. lepida*-like mitochondrial type (Patton *et al.* 2007).

Neotoma cinerea. *Neotoma cinerea* (the bushy-tailed woodrat) is the largest and most cold-tolerant species of *Neotoma*. Its distribution spans a broad latitudinal range that extends from northern New Mexico and Arizona to the arctic regions of Canada (Figure 1). The species primarily occupies montane woodland habitats and builds large middens within cliffs, caves and rock outcrops. The species is characterized by five mitochondrial subclades, several of which show signatures of post-glacial expansion, including the clade sampled herein (INT, Hornsby and Matocq 2012) that is currently found throughout the northern Sierra Nevada and the Great Basin Desert.

The morphological distinction of *N. cinerea* from other woodrats resulted in its early placement in its own genus/subgenus *Teonoma* (Goldman 1910; Burt and Barkalow 1942), but later morphological analyses (Carleton 1980) suggested a close relationship between *N. cinerea* and *N. fuscipes*. Subsequent molecular analyses (Edwards and Bradley 2002; Matocq *et al.* 2007) have, likewise, shown contrasting results concerning the placement of *N. cinerea* within *Neotoma*.

Materials and methods

Sample collection. We collected woodrats representing five species from the following sites (Figure 2; Appendix 1): *N. cinerea* (Cline Buttes, OR, N = 2; Valley Falls, OR, N = 5; Stead, NV, N = 4); *N. fuscipes* (Weed, CA, N = 4; Likely, CA, N = 3; Pilot Hill, CA, N = 4; Soquel, CA, N = 4); *N. macrotis* (Arroyo Seco, CA, N = 4; Erskine Creek, CA, N = 3; Lake Isabella, CA, N = 4; Hungry Valley, CA, N = 4; Caspers Wilderness Park, CA, N = 4); *N. lepida* (Stead, NV, N = 4; Lake Isabella, CA, N = 4); *N. bryanti* (Hungry Valley, CA, N = 3). For the mitogenome analysis we also included samples previously collected by J. L. Patton of *N. bryanti* (Crocker Grade, CA, N = 4; Joaquin Flats, CA, N = 4; Porterville, CA, N = 3) and *N. lepida* (Halloran Spring, CA, N = 4; Pisgah Lava Flow, CA, N = 4). While some sample locations for *N. cinerea* and *N. lepida* occur outside of California, the sampled clades are those that occur in California. Additional specimen and locality data are provided in Appendix 1. All new collections were done in accordance with permits issued by the California Department of Fish and Wildlife, Oregon Game and Fish, and the Nevada Department of Wildlife as well as oversight of the University of Nevada, Reno Institutional Animal Care and Use Committee.

Sequencing and variant calling. From the newly collected specimens, we extracted high quality DNA from liver tissue using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to manufacturer instructions. From the previously collected specimens, we used DNA extractions originally generated by and reported in Patton *et al.* (2007) and loaned to us by the Museum of Vertebrate Zoology. The quantity of the extracted DNA was measured with a Qubit 2.0 Fluorometer (Invitrogen, USA). DNA quality and fragment size distribution was assessed using 1% agarose gel electrophoresis. When necessary, DNA was sheared using a Covaris M220 ultrasonicator (Covaris Inc., USA). Libraries were constructed using the NEBNext® Ultra™ II DNA Library Preparation Kit (New England Biolabs Inc., USA) according to manufacturer instructions. Final libraries had an average insert size of 300 to 400 bp.

Libraries were sequenced by Novogene on an Illumina Novaseq S4 to generate paired-end 2x150 bp sequence data. We removed adapters and quality filtered and trimmed raw DNA sequence reads using the software fastp (Chen *et al.* 2018). We produced a pseudo-chromosomal reference genome by using the program RagTag v2.1 (Alonge *et al.* 2022) to combine the trio-binned, scaffold-scale *Neotoma bryanti* reference genome (https://osf.io/xck3n/; Greenhalgh *et al.* 2022) into chromosome length scaffolds based on the unpublished *Neotoma fuscipes* reference genome (Holding *et al*., forthcoming). We aligned short reads to this reference with BWA-MEM (Li 2013) and piped outputs directly to elPrep for sorting, duplicate marking, and read group replacement using the elPrep filter function (Herzeel *et al.* 2015). This step ensured efficient processing and standardization of the BAM files for downstream analyses. Read group information for each bam file was derived from the input FASTQ files to maintain sample integrity and traceability.

Variant calling was performed using bcftools (Li 2011). First, bcftools mpileup was executed with the parameters -Ou, -B, -C 50, -a QS, AD, and DP to output uncompressed variant data and annotate quality metrics. The mpileup results were input to bcftools call using the -mv and -Oz options for variant calling. The raw VCF file was then filtered using vcftools with minimum depth of four, a maximum depth of 30, a minimum allele frequency of 0.017, a minimum base quality of 30, and a minimum missing variants of 80 %.

Mitogenomes. Whole mitochondrial genomes were extracted from our raw sequencing reads via a custom pipeline. We first used BLAST+ (v.2.9) to query the R1 files from our paired-end read set against the NCBI "mito" database available at https://ftp.ncbi.nlm.nih.gov/blast/db. We retained the top hit for each query, then used the R1 and

matching R2 reads for each hit as input to the program metaSPAdes (Nurk *et al.* 2017). The mitochondrial genome was not recovered for one *N. macrotis* individual from Lake Isabella (UNR 4133); for all other individuals, the longest record in the metaspades.scaffolds.fasta output file corresponded to the full mitochondrial genome. Because the mitochondrial genome is circular, the fasta sequences had to be re-flowed to start and end in the same genomic location before they could be aligned. We re-flowed all sequences to start at tRNA-Phe, which is canonical for Vertebrata, using an internally developed script (https://github.com/calacademy-research/assembly_etc/tree/main/categories/mito). We augmented our dataset using whole mitochondrial genomes previously published on GenBank from three ingroup species (*N. albigula:* NC_068809*; N. magister:* NC_039670*;* and *N. mexicana:* KY707300) and six outgroups: *Onychomys leucogaster* (NC_029760), *Peromyscus californicus* (OP524493), *P. leucogaster* (NC_037180), *P. leucopus* (NC_037180), *P. maniculatus* (NC_039921), and *Reithrodontomys mexicanus* (NC_035597). All mitochondrial genomes were aligned using MAFFT v7 (Katoh and Standley 2013).

Mitochondrial phylogeny and divergence time estimation. We conducted a Bayesian phylogenetic analysis in BEAST v2.7.4 (Bouckaert *et al.* 2014) to reconstruct evolutionary relationships and estimate divergence times from our mitochondrial data. Each coding region in the alignment was treated as a separate partition, and substitution models were estimated for each partition during the analysis using bModelTest (Bouckaert and Drummond 2017); non-coding regions were excluded. To calibrate the root of the tree, we applied a log-normal prior with a hard minimum age of 9.2 million years and a soft maximum (M = 2.0, S = 1.0). This calibration was based on the species *Paronychomys*†, which is an extinct member of the subfamily Neotominae known from fossil deposits that are at least 9.2 million years old (Whistler and Burbank 1992; Kelly and Martin 2022). We then employed an Optimized Relaxed Clock model (Drummond *et al.* 2006) with broad priors applied to branch rates (ORCRates log-normal prior; M = 1, S = 0.5) and branch rate variation (ORCsigma exponential prior; mean = 0.333) to accommodate rate variation among lineages. The clock rate prior (ORCucldMean) followed a log-normal distribution (M = 3.0, S = 0.5), with the mean value being roughly informed by counting the mean pairwise substitutions between ingroup and outgroup species in the dataset and assuming an approximate root age of 10 million years. Bayesian analyses were performed in four independent runs of 100 million generations each, sampling every 5,000 generations. Convergence and mixing were assessed using Tracer v1.7.2 (Rambaut *et al.* 2018), with effective sample size values exceeding 200 used as the threshold for reliable parameter estimates. Two of the runs were excluded due to poor mixing and convergence. We combined the log and tree files from the remaining two runs (200 generations total) using LogCombiner (Drummond and Rambaut 2007), applying a burn-in of 20 % to each file. The maximum clade credibility tree with common ancestor node heights was generated from the combined tree file using TreeAnnotator (Drummond and Rambaut 2007) and was visualized using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Nuclear phylogeny and divergence estimation. We estimated a maximum-likelihood phylogeny from our nuclear dataset using the program IQ-Tree v.2.2.2.7 (Minh *et al.* 2020). As input, we converted our filtered VCF file (see above) to phylip format using the python script vcf2phylip (https://github.com/edgardomortiz/vcf2phylip). We then used the GTR+ASC substitution model, which applies ascertainment bias correction (Lewis 2001) to SNP data, and obtained branch supports using ultrafast bootstrapping (Hoang *et al.* 2018) with 1000 replicates. The final tree was visualized using FigTree v1.4.4.

We computed a matrix of pairwise p-distances among all individuals in our mitochondrial dataset using the function "dist.dna" (model = "raw") in the R package ape (Paradis and Schliep 2019). We also calculated a matrix of p-distances from the nuclear SNP dataset using the command "Dset" (Distance = P) in PAUP*v.4.0a (Swofford 2003). For both matrices, mean p-distances within and among populations were calculated using Excel (Microsoft Corporation).

Estimates of introgression. We used two approaches to detect admixture among the lineages examined herein. We did not include populations close to known areas of active hybridization in this analysis, so we did not anticipate finding hybrid individuals. Nonetheless, we use both population and phylogenetic approaches to identify potential historic admixture between the lineages. To explore population and lineage subdivision in the nuclear dataset, we utilized ANGSD (htslib version 1.17; Korneliussen *et al.* 2014) and NGSAdmix (Skotte *et al.* 2013) as implemented in the ANGSD package. In ANGSD, we generated genotype likelihoods for each individual, then used NGSadmix to explore genetic subdivision across the dataset by examining a range of possible genetic clusters (K) ranging from 1 to 12. To determine support for each value of K we used the method described by Evanno *et al.* (2005), as implemented in Clumpak (Cluster Markov Packager Across K; Kopelman *et al.* 2015).

To identify historic introgression among the lineages, we used a phylogenetic network approach implemented in the PhyloNet package (Than *et al*. 2008; Wen *et al.* 2018). This approach recognizes that a simple model of lineage bifurcation cannot fully capture the process of evolutionary divergence, especially among closely related lineages that are likely to be characterized by incomplete lineage sorting and introgression. We used 10,000 randomly subsampled biallelic SNPs from our filtered VCF file and restricted this dataset to include only one individual per lineage (the individual with the lowest amount of missing data) to reduce computational burden. We explored different maximum numbers of reticulations (MR = 0, 1, and 2) using the *MCMC_Bimarkers* algorithm (Zhu and Nakhleh 2018;

Zhu *et al.* 2018). In each analysis, we used a Markov Chain Monte Carlo (MCMC) with 500,000 generations, sampling every 500 generations after a 200,000 generation burn-in. The "varytheta" flag was used to account for potential differences in population size among the lineages. After running each model (MR = 0-2), we plotted the maximum a posteriori (MAP) score of the best supported network and selected the model that resulted in a sharp improvement in MAP. The best supported network was then visualized using IcyTree (Vaughan 2017).

Results

Mitogenome relationships and divergence timing. The final mitochondrial dataset included 86 individuals and had a total aligned length of 17,734 base pairs (bp). Our phylogenetic reconstruction based on full mitogenomes provides strong support for relationships across the depth of divergences pertinent to the lineages examined here with the majority of nodes supported by Bayesian posterior probabilities >0.95 (Figure 2, 3 ingroup taxa; Supp. Figure 1, full taxon set). The analysis strongly places *N. cinerea* outside of the remainder of *Neotoma*, as represented by *N. albigula*, *N. magister*, *N. mexicana, N. fuscipes*, *N. macrotis*, *N. lepida*, and *N. bryanti*. As expected, *N. albigula* and *N. magister* are more closely related to one another than to *N. mexicana*, consistent with previous analyses (Edwards and Bradley 2002; Matocq *et al.* 2012; Bradley *et al.* 2022). Within the five focal species, several well-supported clades are evident but with minimal geographic structure. Within *N. fuscipes*, there is strong support for a west central clade (represented by the locality Soquel) and a northern clade represented by the localities Pilot Hill, Weed, and Likely, consistent with earlier recognition of this subdivision with *N. fuscipes* (Matocq 2002a). We also recover a distinct subclade of *N. lepida*-like mitochondria in the Porterville population of *N. bryanti*, as expected from the ancient mitochondrial capture discovered by Patton *et al.* (2007) in this population and surrounding region.

Figure 2. Spatial sampling of nuclear and/or mitochondrial diversity across five species of woodrats in California. Note that the Porterville population is depicted with a combination of colors because *N. bryanti* of this population and the southern Sierra Nevada, Tehachapi Mountains, and western Mojave Desert region have a *N. lepida*-like mtDNA.

Our analysis places the base of divergence of *Neotoma* at approximately 4.7 mya (median common ancestor height = 4.71 mya, 95 % CI: 4.27 to 5.29 mya) and the base of the 4 focal lineages *N. fuscipes*, *N. macrotis*, *N. bryanti*, and *N. lepida* at approximately 3.5 mya (median 3.53 mya, 95% CI: 3.18 – 3.97 mya; Figure 3). The subdivision of *N. fuscipes* and *N. macrotis* is estimated to have occurred 2.23 mya (95 % CI: 1.99 to 2.53 mya), followed by subdivision of the clades that include *N. bryanti* and *N. lepida* 1.63 mya (95 % CI: 1.43 to 1.86 mya), and then subdivision of the northern and west central clades of *N. fuscipes* 1.45 mya (95 % CI: 1.27 to 1.67 mya). The haplotypes we sampled within *N. cinerea*, *N. macrotis*, and *N. bryanti* began diverging in the range of 420 to 490 kya, while the individual lineages within *N. fuscipes* and *N. lepida* appear to have diversified more recently (60, 90, and 180 kya).

Across the mitogenome, average uncorrected pairwise sequence divergences within lineages represented by more than one population ranged from 0.4 % in the northern lineage of *N. fuscipes* to 1.1 % in *N. cinerea* (Table 1). The two lineages of *N. fuscipes* differ from one another by 6.3 % mitochondrial sequence divergence. *Neotoma macrotis* differs from the two lineages of *N. fuscipes* by approximately 8.0 to 8.2 %, while *N. bryanti* and *N. lepida* differ from one another by approximately 6.9 %. On average, *N. macrotis*-*N. fuscipes* differ from *N. bryanti*-*N. lepida* by 10.6 to 11.0 %, and these lineages each differ from *N. cinerea* by over 12 %. The distinct subclade of *N. lepida*-like mitochondria in the Porterville population of *N. bryanti* is approximately 1 % divergent from other *N. lepida* mitochondria.

Nuclear phylogeny, diversity and differentiation. The final filtered VCF file contained a total of 342,297 variant sites for 56 individuals, which was used in all downstream analyses. The nuclear phylogenetic analysis (Figure 4) yielded strong support for the relationships among the individuals and taxa for which nuclear genome-wide data was available. In comparison to the mitogenome tree, the nuclear data more consistently recovered locality-specific subclades. Consistent with the mitogenome analysis, we recovered well-differentiated west central and northern clades of *N. fuscipes*.

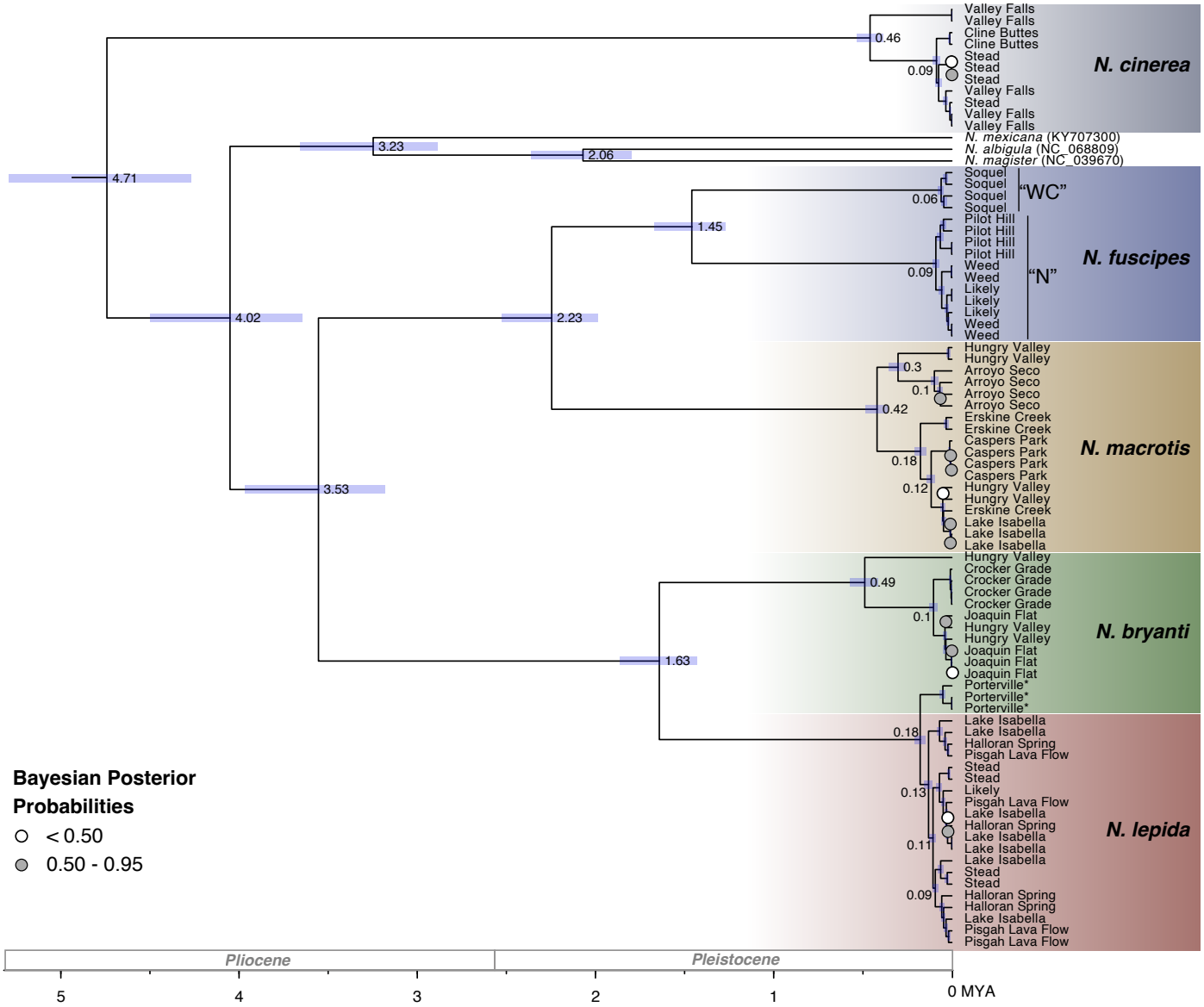

Figure 3. A time-calibrated Bayesian phylogeny estimated using whole mitochondrial genome data in BEAST2. All nodes are supported with > 0.95 Bayesian Posterior Probability except for those shown in white and gray according to the legend. To better visualize focal ingroup taxa divergences, outgroups have been removed but the entire tree is shown in full in Supplemental Figure 1. Nodes are labeled with median ages (in millions of years), with the light blue bars denoting 95 % confidence intervals. Tips from our five focal species are labeled according to collection locality. Additional taxa were included in the analysis and are identified with their GenBank accession numbers. Note that three individuals of *N. bryanti* from the Porterville locality (indicted by *) fall out within the *N. lepida* clade. Two clades within *N. fuscipes* (West-Central, "WC", and North, "N") are labeled and discussed in the text.

Within the northern clade of *N. fuscipes*, the central Sierran population of Pilot Hill clusters separately from the more northerly set of populations, Likely and Weed.

Across the nuclear genome, average uncorrected pairwise sequence divergences within lineages represented by more than one population ranged from 0.6 % in the northern lineage of *N. fuscipes*, *N. lepida* and *N. cinerea* to 1.3 % in *N. macrotis* (Table 1). The two lineages of *N. fuscipes* differ from one another by 5 % nuclear sequence divergence. *Neotoma macrotis* differs from the northern lineage of *N. fuscipes* by approximately 7 % while differing from the west central lineage by 6 %. *Neotoma bryanti* and *N. lepida* differ across the nuclear genome by approximately 6.5 % . On average, *N. macrotis-N. fuscipes* differ from *N. bryanti-N. lepida* by 12.1 to 13.7 %, and these lineages each differ from *N. cinerea* by 17.0 to 18.2 %.

Admixture and introgression. The NGSAdmix analysis showed that the nuclear dataset was most consistent with K = 5 and K = 7, but we show K = 3 to 7 because we find the progression of subdivision informative (Figure 5). At K = 3 we find the expected distinction of *N. cinerea* from an *N. fuscipes-N. macrotis* group and an *N. lepida-N. bryanti* group. At K = 4, *N. fuscipes* and *N. macrotis* become distinct, and interestingly, the Soquel population (representing west central *N. fuscipes*) appears to be admixed between *N. fuscipes* and *N. macrotis*. At K = 5, we see the distinction of the Soquel population of *N. fuscipes* (the most supported solution), at K = 6 we see a poorly supported solution with subdivision within *N. cinerea*, and at K = 7 we see the distinction of *N. bryanti* from *N. lepida* (the

Table 1. Mitogenome (lower half matrix) and nuclear (upper half matrix) pairwise sequence divergence (uncorrected p-distance) within and between *Neotoma* lineages. * denotes the Porterville, CA population of *N. bryanti* that possesses a *N. lepida*-like mitochondrion; the *N. bryanti* values do not include the mismatched population.

	***N. fuscipes* N**	***N. fuscipes* WC**	***N. macrotis***	***N. bryanti***	***N. bryanti****	***N. lepida***	***N. cinerea***
N. fuscipes N	0.004/0.006	0.050	0.071	0.136	-	0.137	0.182
N. fuscipes WC	0.063	0.004/0.004	0.059	0.124	-	0.127	0.170
N. macrotis	0.080	0.082	0.015/0.013	0.121	-	0.125	0.171
N. bryanti	0.107	0.106	0.104	0.008/0.003	-	0.065	0.179
*N. bryanti**	0.108	0.110	0.107	0.069	0.002/-	-	-
N. lepida	0.108	0.109	0.106	0.069	0.010	0.006/0.006	0.179
N. cinerea	0.121	0.122	0.124	0.125	0.123	0.122	0.011/0.006

second most supported solution).

Using PhyloNet, we tested whether a phylogenetic network (allowing for reticulation) offered a better fit to our data compared to a traditional bifurcating tree. We tested models with a maximum of 0, 1, or 2 reticulations (MR = 0 to 2) and found a significant improvement in model MAP scores at MR = 1 (Posterior ESS = 174; Figure 6). The network topology suggests a sister relationship between *N. cinerea* and the *N. macrotis*-*N. fuscipes* clade, but with ancestral introgression between the ancestors of *N. macrotis*-*N. fuscipes* and the ancestors of *N. lepida*-*N. bryanti* (inheritance probability $\gamma = 0.47$).

Discussion

The woodrats of California occupy a wide range of native habitats and their diversification through time provides insight into the evolutionary processes that have led to the accumulation and retention of biodiversity across these landscapes. We use genome-wide data to gain insight into patterns of diversity at various spatial scales to establish a baseline from which to conduct further analyses to identify processes that generate and maintain diversity across space and time in this system.

Mitochondrial relationships in western Neotoma. Evolutionary relationships within *Neotoma* and its allies have been examined using morphological characters, and both mitochondrial and nuclear markers (reviewed in Edwards and Bradley 2002; Matocq *et al.* 2007; and Bradley *et al.* 2022). All recent molecular analyses are consistent with the sister relationship between *N. fuscipes* and *N. macrotis*, and of the close relationship between *N. lepida* and *N. bryanti* (Patton *et al.* 2007; Matocq *et al.* 2007; Bradley *et al.* 2022). Likewise, the representative other lineages of woodrats included here – *N. albigula, N. magister* and *N. mexicana* – consistently group outside the lineage that contains *N. fuscipes*, *N. macrotis*, *N. lepida* and *N. bryanti*. Nonetheless, one of the outstanding questions with regards to the western woodrat lineages examined here is the placement of *N. cinerea*. As previously mentioned, the morphological distinction of *N. cinerea* resulted in this species being placed in its own genus/subgenus *Teonoma* (Goldman 1910; Burt and Barkalow 1942), but later morphological analyses (Carleton 1980) suggested a closer relationship with *N. fuscipes*. The distinction of *N. cinerea* is also reflected in molecular data and the fact that *N. cinerea* is characterized by a "long branch" of evolutionary change, a particularly difficult problem when using parsimony analysis (Felsenstein 1978). Matocq *et al.'s* (2007) parsimony analysis of two mitochondrial and 4 nuclear loci weakly placed *N. cinerea* as sister to the rest of *Neotoma*, consistent with previous work by Edwards and Bradley (2002) based on cytochrome-*b* sequence data. However, Matocq *et al.'s* (2007) Bayesian analysis placed *N. cinerea* within *Neotoma*, as sister to the clade containing *N. macrotis* and *N. fuscipes*. The most recent and comprehensive analysis of *Neotoma* and its close allies based on Bayesian analysis of five nuclear loci and four mitochondrial genes found strong support that *N. cinerea* belongs within the clade containing *N. macrotis*, *N. fuscipes*, *N. lepida*, and *N. bryanti* (Bradley *et al.* 2022).

Our Bayesian analysis of the full mitogenome of select representatives of *Neotoma* again places *N. cinerea* outside the remainder of *Neotoma*. Because a nested position for *N. cinerea* is recovered in past analyses that have included both nuclear and mitochondrial loci (*e. g.,* Matocq *et al.* 2007 and Bradley *et al.* 2022) as well as in our phylogenetic network analysis of genome-wide nuclear data, it seems clear that the mitochondrial history of *N. cinerea* is distinct from that recorded by its nuclear genome. One possible cause of this discrepancy is that the long branch of evolutionary divergence that characterizes the mtDNA of *N. cinerea* may be due to higher rates of mitochondrial evolution, perhaps in part due to selection (Ballard and Rand 2005). The cold climates occupied by *N. cinerea* may pose unique physiological challenges that have imposed selection on the mitochondrial genome to meet the energetic demands of low or fluctuating temperature environments (Breton *et al.* 2021). Mitochondria play a central role in oxidative phosphorylation and thermogenesis (Cannon and Nedergaard 2004) and specific mtDNA haplotypes have been shown to be associated with higher thermogenesis in mammals (Fontanillas *et al.* 2005). Further geographic and genomic sampling across the large climatic gradient occupied by *N. cinerea* is needed to provide the basis from which to gain insight into the history of environmental selection and adaptive evolution in this lineage.

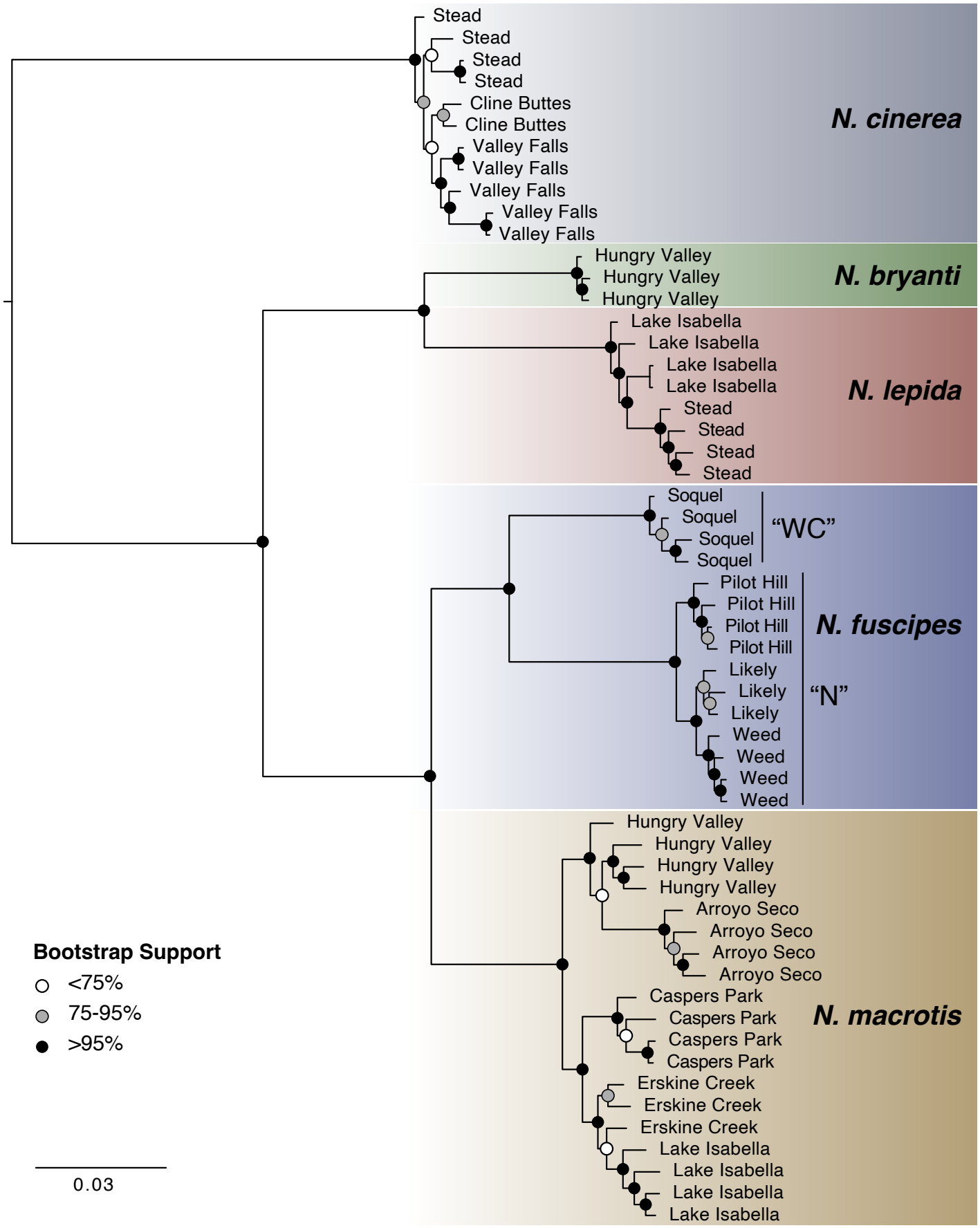

Figure 4. Maximum-likelihood phylogeny estimated using nuclear SNP data. Nodes with low (<75 %), medium (75 to 95 %), or high (>95 %) bootstrap support are indicated using white, gray, or black circles, respectively. Branch lengths are scaled to substitutions per site. Tips are labeled according to collection locality. The topology we present is mid-point rooted on the longest branch. Two clades within *N. fuscipes* (West-Central, "WC", and North, "N") are also labeled and discussed in the text.

Timing and geographic context of divergence in western lineages of Neotoma. According to our mitogenome analysis, the first major diversification in the clade that includes *N. macrotis*, *N. fuscipes*, *N. lepida*, and *N. bryanti* occurred 3.5 mya during the Pliocene (which spanned ~5 to 2.5 mya). The fossil record is not adequately detailed to provide insight into where the major divergence occurred that led to the ancestors of *N. macrotis-N. fuscipes* and the clades containing *N. lepida* and *N. bryanti*. However, this divergence likely occurred at a time depth when major landscape changes were still occurring, especially across southern California, such as the continued uplift of the Transverse Range, Tehachapi Mountains, and Peninsular Range, and major water embayments along the southern coast of California and northern Baja California, including an inundated southern Central Valley (Hall 2002; Jacobs *et al.* 2004; Peryam *et al.* 2011). Such major landscape changes likely contributed to early isolation and differentiation of the ancestors of the modern lineages.

Patton *et al.* (2007) proposed that the two lineages containing *N. bryanti* and *N. lepida*, respectively, diverged from one another approximately 1.6 mya (highly consistent with our full mitogenome estimate of 1.63 mya) in southern California as a result of reduced connectivity across the floodplain habitats of the Pliocene Bouse lake/embayment (their figure 146). For *N. fuscipes* and *N. macrotis,* Matocq (2002a; 2002b) proposed that the species diverged due to north-south separation by glacial rivers along the central Sierra Nevada, approximately 2 mya, which our full mitogenome estimate pushes to 2.23 mya. To reconcile the timing and proposed spatial position of these more recent divergences with the deeper divergence that led to these lineages (3.5 mya), we propose that the geologically active region of the western Transverse Range and Tehachapi Mountains may have been the site of initial divergence. That is, the ancestors of *N. fuscipes-N. macrotis-N. bryanti-N. lepida* could have been broadly distributed across this portion of southern California during the climatic optimum of the early Pliocene (4.5 to 3.5 mya), but when these warmer conditions were replaced by cooler and more variable conditions (Peryam *et al*. 2011), distributional shifts could have been coupled with lineage differentiation. Specifically, under cooler and more variable conditions, the ancestors of *N. lepida-N. bryanti* would have partly retreated south and further diversified across the modified habitats of the previous Bouse lake/embayment. The ancestors of the more northern lineage, *N. fuscipes-N. macrotis*, would have meanwhile primarily occupied areas north of the Transverse-Tehachapi region and experienced subsequent differentiation due to water and glacial barriers in the Central Valley and Sierran foothills, including the differentiation of the northern and west central clades of *N. fuscipes* (1.45 mya). We note that this geographic scenario could easily encompass *N. cinerea* as part of the early divergence of this western clade of woodrats by adding a lineage that largely expanded east and north.

The hypothesis that the Transverse-Tehachapi region would have played a role in the early divergence of the western lineages of woodrats is consistent with the known role of this region in generating and maintaining biodiversity (Davis *et al.* 2008). It is likely that the sharp elevational relief of this region has amplified climatic fluctuations through time (Badgley *et al.* 2017) and created repeated pulses of isolation among populations that retreated into nearby lowlands. In fact, even within *Neotoma macrotis*, we find relatively shallow, but spatially distinct lineages that come into contact in the Transverse-Tehachapi region; a pattern consistent in both the mitochondrial and nuclear datasets. One lineage appears to extend along the southern Coast Ranges to the western portion of the Transverse-Tehachapi region (localities Arroyo Seco and Hungry Valley), while the other extends from the southern Sierra Nevada and Tehachapi Mountains (Lake Isabella and Erskine Creek) into southern California (Caspers Wilderness Park), with the mitogenome data showing haplotype sharing in the Hungry Valley locality in the Transverse-Tehachapi region. These subclades are spatially consistent with those documented by Matocq (2002a). Overall, the Transverse-Tehachapi region is well known as a site of biogeographic subdivision and confluence among species/lineages with more western/northern distributions and those with more southern/eastern distributions (reviewed in Gottscho 2016). The confluence (and perhaps repeated confluence) of closely related lineages across this region may have contributed to further ecological divergence, but also allowed genetic admixture (Davis *et al.* 2008); both processes contributing to high levels of diversity, and both being clearly reflected at various evolutionary depths in the woodrats of this region.

Hybridization in the history of western Neotoma. As each species of woodrat has continued to expand (and perhaps re-expand) into their current distributions, one evolutionary phenomenon that has repeatedly occurred is hybridization between each set of closely related lineages when they come into secondary contact. Throughout California, there are multiple examples of recent and ongoing hybridization between *N. lepida* and *N. bryanti*, as well as between *N. fuscipes* and *N. macrotis* (detailed below). Moreover, our phylogenetic network analysis suggests ancient hybridization at deeper evolutionary timescales. The phylogenetic network showed support for the placement of *N. cinerea* as sister to the clade containing *N. fuscipes* and *N. macrotis*, but that this topology requires inference of introgression between the ancestors of *N. fuscipes-N. macrotis* and those of *N. lepida-N. bryanti*. While these results are preliminary, they serve as a reminder that the propensity of these animals to interbreed is unlikely to be restricted to the modern lineages and that ancient introgression events should be considered in our reconstruction of woodrat evolutionary history.

*Neotoma bryant*i and *N. lepida*. The most impressive example of historic hybridization has led to a pattern of mitonuclear mismatch that extends throughout the south-

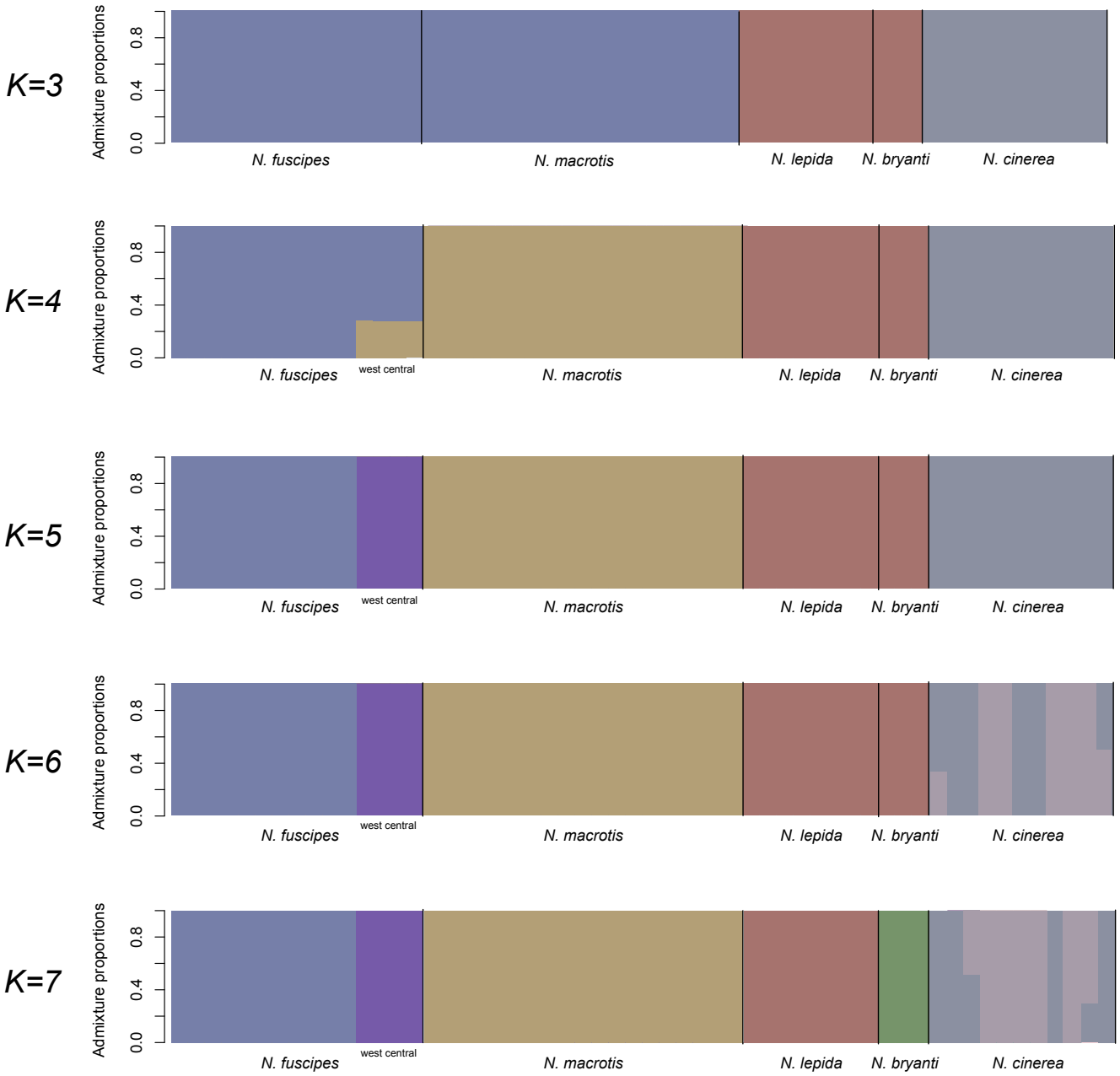

Figure 5. Genomic composition of 56 individual woodrats representing five species of woodrats. K of five and seven received the strongest support.

ern Sierra Nevada and Tehachapi Mountains (Patton *et al.* 2007), again demonstrating the importance of this region in the history of western woodrat lineages. After their initial divergence, the current mtDNA diversity in *N. bryanti* suggests that they expanded north approximately 0.5 mya (Figure 3; Patton *et al.* 2007). *Neotoma lepida* likely expanded north more recently; 0.3 to 0.4 mya according to Patton *et al.* (2007) and 0.13 to 0.18 mya in the small geographic extent we sampled here, and depending on whether we include the *N. bryanti*-captured clade (Figure 3). Once the lineages met during their northern expansion, likely in the vicinity of the Transverse-Tehachapi region, they hybridized (female *N. lepida* and male *N. bryanti*) which led to descendants with purely *N. bryanti* nuclear genomes and a *N. lepida*-like mtDNA. Interestingly, while active hybridization between *N. bryanti* and *N. lepida* occurs nearby in the west Mojave Desert (Patton *et al.* 2007; Shurtliff *et al.* 2014; Jahner *et al.* 2021; Nielsen *et al.* 2021 and 2023), laboratory mate choice trials suggest that most modern interspecific pairings are likely between *N. bryanti* females and *N. lepida* males (Shurtliff *et al.* 2013). Nonetheless, even if the reciprocal cross is relatively rare, this rare event clearly had a profound evolutionary consequence. The current pattern of spatial mitonuclear mismatch is consistent with multiple demographic scenarios (Patton *et al.* 2007), however, the spread and maintenance of this novel recombinant type under any scenario would have been facilitated if it were favored by selection. Whether the novel *N. lepida*-like mtDNA could impart a physiological advantage over the *N. bryanti* mitochondrial type remains to be investigated (K. Everson, forthcoming).

Neotoma fuscipes and *N. macrotis*. Historic hybridization and mitochondrial capture has also been documented between *N. fuscipes* and *N. macrotis*. Such admixed ancestry characterizes the animals that occupy Caswell Memorial State Park and nearby locations (Matocq *et al.* 2012). In these now isolated populations, both *N. fuscipes* and *N. macrotis* mitochondrial types co-occur, and at least based on nuclear microsatellite analysis, the nuclear genomes appear to be of admixed ancestry between the two species (Matocq *et al.* 2012). Although such known admixed populations were not included in our analysis here, we were intrigued to see that at K = 4, just prior to the west central clade of *N. fuscipes* (represented by Soquel) emerging as a distinct genetic unit at K = 5 (purple set, Figure 5), that the Soquel individuals appeared partially admixed between *N. fuscipes* and *N. macrotis*. Although preliminary, these results may suggest that interspecific hybridization that led to the admixed population(s) in the Central Valley may not be restricted to that region alone and may extend to the eastern and western flanks of the valley perhaps from a time when east-west habitat continuity was much greater across this region (reviewed in Matocq *et al.* 2012).

Ongoing hybridization between *N. macrotis* and *N. fuscipes* occurs where their ranges meet in the southern Coast Ranges along the Nacimiento River south of Monterey Bay (Coyner *et al.* 2015; Hunter *et al.* 2017; Matocq *et al.* 2024). During a five-year period, the center of the hybrid zone shifted in part due to the small-bodied *N. macrotis* having a survival advantage over the larger-bodied *N. fuscipes* in years with dry winters (Hunter *et al.* 2017). This survival advantage coupled with dispersal of *N. macrotis* into the range previously dominated by *N. fuscipes* led to augmented interspecific mating opportunities and an increase in admixture over time (Matocq *et al.* 2024). This system will continue to provide novel insight into the important link between weather/climate fluctuations and rates of hybridization and the potential for interspecific gene flow.

Mechanisms contributing to species boundaries. Patterns of hybridization between *N. fuscipes* and *N. macrotis*, as well as between *N. lepida* and *N. bryanti* suggest that both pre- and post-zygotic isolating mechanisms impact realized gene flow between these lineages. The site of secondary contact and hybridization between *N. lepida* and *N. bryanti* at Whitney Well in the west Mojave Desert is characterized by a sharp ecotone that helps maintain fine-scale parapatry between the two species creating a pre-zygotic filter to gene flow due to relatively infrequent interspecific mating opportunities (Shurtliff *et al.* 2014; Nielsen *et al.* 2021, 2023). Such an ecological filter to hybridization, though, does not occur at another site of hybridization between these two lineages (Klure *et al.* 2023) nor is there an obvious ecological component to the active hybrid zone between *N. macrotis* and *N. fuscipes* (Coyner *et al.* 2015). In both pairs of taxa, when interspecific mating opportunities arise, body size (and perhaps associated levels of aggression) appears to play a role in mating decisions, where females of the smaller-bodied congener (*N. macrotis* and *N. lepida*) rarely choose to mate with males of the larger-bodied species (*N. fuscipes* and *N. bryanti*, respectively; Shurtliff *et al.* 2013; Matocq *et al.* 2024). As such, in both systems, the generation of F1s is asymmetric in terms of who serves as the mother species and father species, yet genomic patterns clearly show that backcrossing occurs in both parental directions, which would allow for introgression between the parental species (Shurtliff *et al.* 2014; Coyner *et al.* 2015). Nonetheless, both study systems also show evidence of

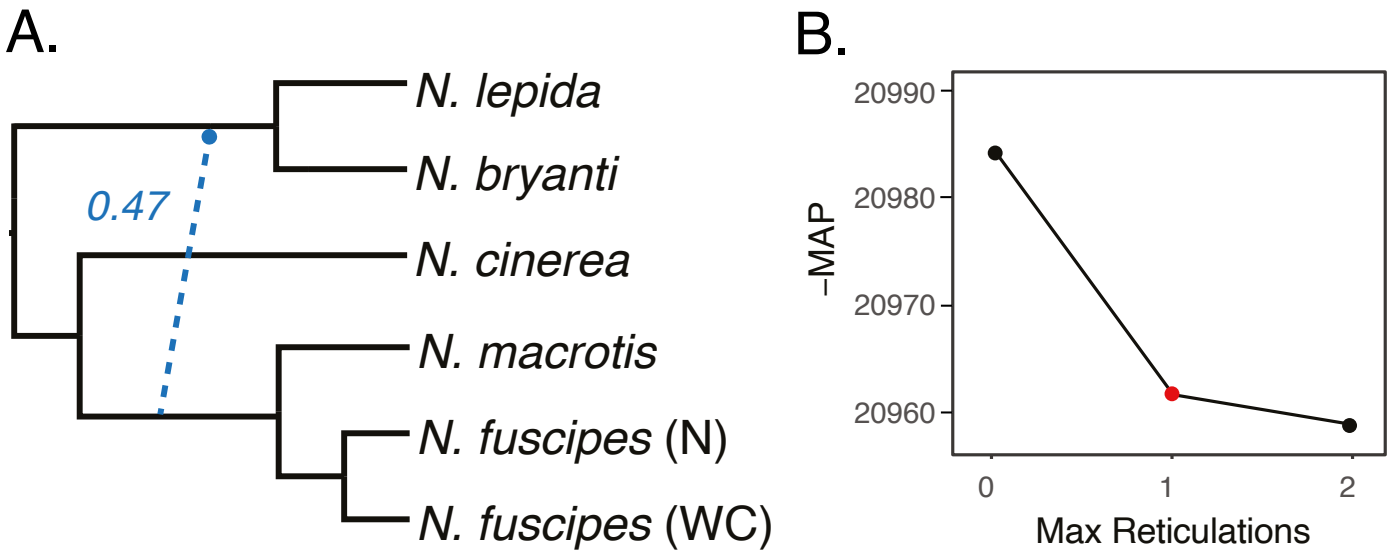

Figure 6. Results of a phylogenetic network analysis performed using PhyloNet (Wen *et al.* 2008) with one representative individual from each of the focal lineages. A) The best-supported phylogenetic network with a maximum of one reticulation (MR = 1). The blue dotted line depicts the inferred introgression event (inheritance probability: γ = 0.47). B) Negative MAP scores of the best models estimated using MR = 0 - 2. The MR = 1 model was selected (shown in panel A) due to the large improvement in score from MR = 0 to MR = 1.

genomic incompatibilities. Specifically, based on genome-wide patterns of variation (*N. lepida* and *N. bryanti*, Jahner *et al.* 2021) and field-measured reproductive success (*N. fuscipes* and *N. macrotis*, Matocq *et al.* 2024), the vast majority of admixed individuals have at least one pure parent, that is, they are the result of backcrossing. This suggests that the genomic stability of admixed individuals requires at least one full parental complement of the genome. Further evidence of genomic incompatibility comes from the *N. fuscipes*/*N. macrotis* hybrid zone where F1 males sire very few young, consistent with Haldane's Rule (Haldane 1922). Finally, while we know that each species pair differ in male genital morphology (Matocq 2002b; Patton *et al.* 2007; Matocq *et al.* 2012), the role of possible genital "mismatch" in fertilization efficiency has yet to be quantified in woodrats. In sum, these woodrat systems provide ample opportunity to continue investigating the role of behavior, morphology, ecology, and genetics in determining the nature of species boundaries and the potential of hybridization and introgression to influence the evolutionary trajectory of these lineages.

Future directions. We are clearly in the earliest stages of our exploration of genome-wide datasets in these lineages, but we see great promise in our ability to address fundamental ecological and evolutionary questions in new ways with such datasets. As genome-wide data continue to become available in these species and across the genus *Neotoma*, we will gain further insight into the timing of important divergence events. Perhaps more importantly, we will gain insight into how different parts of these genomes responded in distinct ways to neutral and selective processes as these species experienced changing environmental conditions through time. These augmented datasets coupled with advances in demographic modeling will provide insight into how these lineages have expanded and contracted across these landscapes and how they have interacted with their environments and each other in the process.

The availability of active hybrid zones between these lineages provides unique laboratories in which to further understand the mechanisms that underlie the causes and consequences of hybridization, including the adaptive leaps that may be possible when a species can "shop" in the genome of a closely related but differentially adapted species. Detailed field studies coupled with thorough genomic sequencing will allow unprecedented insight into the early stages of introgression and how, essentially, one genome is filtered against the background of another through recombination and selection. We are especially interested to follow this process for the parts of the genome that we already know play a significant role in ecological adaptation for these species, including those involved in detoxification (Dearing *et al.* 2005; Greenhalgh *et al.* 2024). We expect that detoxification loci are under strong selection in this system as evidenced by massive expansion of certain detoxification gene families (Greenhalgh *et al.* 2022; Holding *et al.* forthcoming). Identifying the functional significance and evolution of structural variation in these and other gene families will require integration of functional assays with an understanding of how this variation is distributed from individual genomes to a landscape scale that encompasses the environmental variation these species experience. What we know for certain is that the most important discoveries yet to be made in this system and others will come from thorough integration of field and genetic studies conducted across multiple temporal and spatial scales—the hallmark of Jim Patton's approach to studying mammalian evolution.

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Appendix 1

Specimens included in this study. UNR = University of Nevada, Reno; MVZ = Museum of Vertebrate Zoology.

Species	Specimen No.	DNA ID	Site name	County	State	Latitude	Longitude
N. bryanti	UNR 4175	GG18	Hungry Valley SRA	Ventura	CA	34.74479	-118.86694
N. bryanti	UNR 4178	GG21	Hungry Valley SRA	Ventura	CA	34.73934	-118.89086
N. bryanti	UNR 4179	GG22	Hungry Valley SRA	Ventura	CA	34.73827	-118.89222
N. bryanti	MVZ 195975	-	Crocker Grade	San Luis Obispo	CA	35.2	-119.7
N. bryanti	MVZ 195976	-	Crocker Grade	San Luis Obispo	CA	35.2	-119.7
N. bryanti	MVZ 195979	-	Crocker Grade	San Luis Obispo	CA	35.2	-119.7
N. bryanti	MVZ 195980	-	Crocker Grade	San Luis Obispo	CA	35.2	-119.7
N. bryanti	MVZ 196822	-	Joaquin Flat	Kern	CA	35.02651	-118.69642
N. bryanti	MVZ 196823	-	Joaquin Flat	Kern	CA	35.02651	-118.69642
N. bryanti	MVZ 196827	-	Joaquin Flat	Kern	CA	35.02651	-118.69642
N. bryanti	MVZ 198594	-	Joaquin Flat	Kern	CA	35.02651	-118.69642
N. bryanti	MVZ 196074	-	Porterville	Tulare	CA	35.89183	-118.91687
N. bryanti	MVZ 196075	-	Porterville	Tulare	CA	35.89183	-118.91687
N. bryanti	MVZ 196076	-	Porterville	Tulare	CA	35.89183	-118.91687
N. cinerea	UNR 4124	GG23	Cline Buttes	Deschutes	OR	44.25344	-121.27355
N. cinerea	UNR 4125	GG24	Cline Buttes	Deschutes	OR	44.24828	-121.27860
N. cinerea	UNR 4140	GG17	Stead Archery Range	Washoe	NV	39.70629	-119.82730
N. cinerea	UNR 4139	GG31	Stead Archery Range	Washoe	NV	39.70662	-119.82594
N. cinerea	UNR 4145	GG32	Stead Archery Range	Washoe	NV	39.70662	-119.82594
N. cinerea	UNR 4134	GG33	Stead Archery Range	Washoe	NV	39.70656	-119.82590
N. cinerea	UNR 4136	GG39	Valley Falls	Lake	OR	42.48959	-120.35621
N. cinerea	UNR 4135	GG40	Valley Falls	Lake	OR	42.49357	-120.35792
N. cinerea	UNR 4127	GG41	Valley Falls	Lake	OR	42.48955	-120.35680
N. cinerea	UNR 4126	GG42	Valley Falls	Lake	OR	42.50789	-120.34636
N. cinerea	UNR 4128	GG43	Valley Falls	Lake	OR	42.50769	-120.34607
N. fuscipes	UNR 4131	GG35	Likely	Modoc	CA	41.21723	-120.47828
N. fuscipes	UNR 4132	GG36	Likely	Modoc	CA	41.22589	-120.44794
N. fuscipes	UNR 4130	GG37	Likely	Modoc	CA	41.22701	-120.44466
N. fuscipes	UNR 4137	GG2	Pilot Hill	El Dorado	CA	38.82865	-120.99421
N. fuscipes	UNR 4138	GG3	Pilot Hill	El Dorado	CA	38.82891	-120.99425
N. fuscipes	UNR 4276	GG29	Pilot Hill	El Dorado	CA	38.82915	-120.99461
N. fuscipes	UNR 4277	GG30	Pilot Hill	El Dorado	CA	38.82889	-120.99493
N. fuscipes	UNR 4162	GG4	Soquel	Santa Cruz	CA	37.07701	-121.93135
N. fuscipes	UNR 4163	GG5	Soquel	Santa Cruz	CA	37.07732	-121.93193
N. fuscipes	UNR 4164	GG6	Soquel	Santa Cruz	CA	37.07755	-121.92817
N. fuscipes	UNR 4165	GG7	Soquel	Santa Cruz	CA	37.08698	-121.93285
N. fuscipes	UNR 4144	GG44	Weed	Siskiyou	CA	41.53488	-122.24864
N. fuscipes	UNR 4143	GG45	Weed	Siskiyou	CA	41.53424	-122.24792
N. fuscipes	UNR 4141	GG46	Weed	Siskiyou	CA	41.53463	-122.24852
N. fuscipes	UNR 4142	GG47	Weed	Siskiyou	CA	41.53414	-122.24788
N. lepida	UNR 4123	GG48	Lake Isabella	Kern	CA	35.64538	-118.21299
N. lepida	UNR 4120	GG50	Lake Isabella	Kern	CA	35.59020	-118.23845
N. lepida	UNR 4117	GG51	Lake Isabella	Kern	CA	35.76450	-118.08729
N. lepida	UNR 4115	GG57	Lake Isabella	Kern	CA	35.76403	-118.08239
N. lepida	UNR 4271	GG1	Stead Archery Range	Washoe	NV	39.70629	-119.82964
N. lepida	UNR 4270	GG34	Stead Archery Range	Washoe	NV	39.70519	-119.83002
N. lepida	UNR 4119	GG59	Stead Archery Range	Washoe	NV	39.70572	-119.82931

N. lepida	UNR 4129	GG60	Stead Archery Range	Washoe	NV	39.70501	-119.83032
N. lepida	MVZ 215584	-	Halloran Spring	San Bernardino	CA	39.40230	-115.89977
N. lepida	MVZ 215585	-	Halloran Spring	San Bernardino	CA	39.40230	-115.89977
N. lepida	MVZ 215586	-	Halloran Spring	San Bernardino	CA	39.40230	-115.89977
N. lepida	MVZ 215588	-	Halloran Spring	San Bernardino	CA	39.40230	-115.89977
N. lepida	MVZ 215603	-	Pisgah Lava Flow	San Bernardino	CA	34.74704	-116.34514
N. lepida	MVZ 215605	-	Pisgah Lava Flow	San Bernardino	CA	34.74704	-116.34514
N. lepida	MVZ 215607	-	Pisgah Lava Flow	San Bernardino	CA	34.74704	-116.34514
N. lepida	MVZ 215608	-	Pisgah Lava Flow	San Bernardino	CA	34.74704	-116.34514
N. macrotis	UNR 4272	GG25	Arroyo Seco	Monterey	CA	36.23306	-121.48155
N. macrotis	UNR 4273	GG26	Arroyo Seco	Monterey	CA	36.41572	-121.32047
N. macrotis	UNR 4274	GG27	Arroyo Seco	Monterey	CA	36.23420	-121.48046
N. macrotis	UNR 4275	GG28	Arroyo Seco	Monterey	CA	36.23445	-121.48061
N. macrotis	UNR 4169	GG10	Caspers Wldr. Park	Orange	CA	33.54062	-117.55787
N. macrotis	UNR 4167	GG11	Caspers Wldr. Park	Orange	CA	33.55135	-117.56711
N. macrotis	UNR 4166	GG8	Caspers Wldr. Park	Orange	CA	33.54383	-117.55883
N. macrotis	UNR 4168	GG9	Caspers Wldr. Park	Orange	CA	33.54062	-117.55789
N. macrotis	UNR 4172	GG14	Erskine Creek	Kern	CA	35.57532	-118.41425
N. macrotis	UNR 4173	GG15	Erskine Creek	Kern	CA	35.57532	-118.41425
N. macrotis	UNR 4174	GG16	Erskine Creek	Kern	CA	35.57328	118.41571
N. macrotis	UNR 4170	GG12	Hungry Valley SRA	Ventura	CA	34.76903	118.88293
N. macrotis	UNR 4171	GG13	Hungry Valley SRA	Ventura	CA	34.76903	118.88293
N. macrotis	UNR 4176	GG19	Hungry Valley SRA	Ventura	CA	34.74509	118.86663
N. macrotis	UNR 4177	GG20	Hungry Valley SRA	Ventura	CA	34.74607	118.86717
N. macrotis	UNR 4146	GG54	Lake Isabella	Kern	CA	35.76286	118.08183
N. macrotis	UNR 4133	GG55	Lake Isabella	Kern	CA	35.76350	118.08179
N. macrotis	UNR 4148	GG56	Lake Isabella	Kern	CA	35.76271	118.08214
N. macrotis	UNR 4147	GG58	Lake Isabella	Kern	CA	35.76271	118.08215

Supplementary material

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The components of predation in Culpeo Foxes (*Lycalopex culpaeus*), and the value of long-term observations

Douglas A. Kelt[1]*, Peter L. Meserve[2], M. Andrea Previtali[3], W. Bryan Milstead[4], Brian K. Lang, Hector Véas[5, 6], Julio R. Gutiérrez[6], Alejandra J. Troncoso[6, 7], and Madan K. Oli[8]

[1] Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, California 95616 USA. Email: dakelt@ucdavis.edu (DAK).
[2] Department of Biological Sciences, University of Idaho, Moscow, Idaho USA. Email: pmeserve@uidaho.edu (PLM).
[3] Departamento de Ciencias Naturales, Universidad Nacional del Litoral, Santa Fe, Argentina. Email: andrea.previtali@gmail.com (MAP).
[4] U.S. Environmental Protection Agency, National Health and Environmental Effects Laboratory, Atlantic Ecology Division, Narragansett, RI 02882, USA. Email: willbmisled@gmail.com (WBM).
[5] Bernalillo, New Mexico, USA (BKL).
[6] Departamento de Biología, Universidad de La Serena, La Serena, Chile, and Centro de Estudios Avanzados en Zona Arida (CEAZA), La Serena, Chile (JRG). Email: hector.veas@gmail.com (HV), matroncoso@userena.cl (AJT).
[7] Instituto de Ecología y Biodiversidad (IEB), Santiago Chile (JRG). Email: matroncoso@userena.cl (AJT)
[8] Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32611 USA. Email: olim@ufl.edu (MKO).
*Corresponding author: https://orcid.org/0000-0001-8254-9117.

Understanding predator-prey dynamics requires insight on predator responses to variation in prey abundance. Whereas most predators respond numerically to changes in food availability, functional responses are less clear. Characterizing both is essential to understanding how predator-prey dynamics will change with spatiotemporal variation in resources or habitat conditions. The Culpeo (*Lycalopex culpaeus*) is a wide-ranging South American canid broadly characterized as a generalist forager exhibiting numerical responses, but limited functional responses, to variation in prey availability. We employ a 23-year perspective on the diet of Culpeos, using monthly demographic monitoring of small mammal prey species and concurrent collection of Culpeo scat in a large protected area in Chile. As elsewhere, Culpeos emphasize small mammals in their diet, but they consistently consume some species (notably *Abrocoma bennettii*) disproportionate to their apparent availability. Culpeos here display limited numerical responses to variation in small mammal abundance, although this weakens during extended periods of low small mammal availability when foxes presumably switch to other food items. Culpeos exhibit an asymptotic (Type II) functional response to variation in abundance of small mammal prey. While Type II functional responses are generally considered to characterize specialist predators, these patterns match expectations for a generalist forager that strongly favors certain prey species, and underscore the importance of long-term data for elucidating fundamental ecological patterns. Further work is needed to dissect this functional response among key prey species, and to determine if and how this may fluctuate with climatic conditions, which vary extensively here due to El Niño Southern Oscillations. Long-term datasets provide unique opportunities to understand and characterize such patterns in natural communities.

Entender la dinámica depredador-presa requiere comprender cómo los depredadores responden a las variaciones en la abundancia de presas. Mientras la mayoría de los depredadores responderán numéricamente a los cambios en la disponibilidad de alimento, las respuestas funcionales son menos claros. Caracterizando ambas respuestas es esencial para comprender cómo cambiara la dinámica entre depredador y presa a la variación espaciotemporal en recursos o en las condiciones del hábitat. El Culpeo (*Lycalopex culpaeus*) es un cánido sudamericano ampliamente distribuido y generalmente caracterizado como un depredador generalista exhibiendo respuestas numéricas, pero limitada (o no) respuestas funcionales, a la variación en la disponibilidad de presas. Nosotros empleamos una perspectiva de 23 años en la dieta de Culpeo, usando un programa de monitoreo demográfico mensual de micromamíferos y la colección simultánea de fecas de culpeo en un área protegida en Chile. Como en otras partes, los culpeos seleccionan micromamíferos en su dieta, pero consumen algunas especies (especialmente *Abrocoma bennettii*) en forma desproporcionado en relación con su disponibilidad aparente. Culpeos acá muestran una respuesta numérica limitada a la variación en abundancia de micromamíferos, aunque esto es más débil durante largos períodos de disponibilidad baja de micromamíferos cuando los zorros probablemente cambian a otros alimentos. Los culpeos exhiben una respuesta funcional asintótica (tipo II) a la variación en la abundancia de presas de micromamíferos. Tanto como respuestas funcionales tipo II son generalmente considerado a caracterizar depredadores especialistas, estos patrones coinciden con la expectativa de un forrajeo generalista que fuertemente favorece ciertas especies de presa y destacan la importancia de los datos a largo plazo para elucidar patrones ecológicos fundamentales. Se necesitan más trabajos para disectar esta respuesta funcional entre especie de presas clave y para determinar si y cómo esto puede fluctuar con las condiciones climáticas, que pueden variar ampliamente en asociación con la Oscilación del Sur El Niño. Conjuntos de datos a largo plazo proporcionan oportunidades únicas para entender y caracterizar estos patrones fundamentales en las comunidades naturales.

Keywords: Diet; foraging ecology; functional response; generalist predator; long-term data, numerical response.

Introduction

In simplest terms, predator foraging theory predicts that predators should respond to variation in prey availability numerically and/or functionally (*e. g.*, changes in numbers of predators versus changes in per-capita prey consumption—Holling 1959; Jaksic *et al.* 1993). Numerical responses may occur over the short term (immigration and emigration) or longer term (local recruitment or mortality), whereas functional responses are more immediate. Because generalist predators are likely to switch prey and to develop search images for favored prey as they increase in abundance, their functional responses should be sigmoidal in shape (*e. g.*, Type III), with a lagged response as prey numbers increase, followed by increased per capita consumption to some point of satiation. Specialist predators, on the other hand, switch prey less frequently (or among fewer target prey) and are more likely to exhibit Type II functional responses (*e. g.*, with no lag period). However, these functional responses comprise points on a continuum, and we should anticipate variation in the extent to which predators match these patterns (Denny 2014). In general, smaller predators tend to be more generalist and opportunistic in their diet than larger predators, which tend to forage on more prey species but more selectively (Gittleman 1985).

Small canids tend to be opportunistic foragers, and may exhibit either specialist or generalist prey selection. Arctic Foxes (*Vulpes lagopus*) in Siberia act as specialists on Norwegian Lemmings (*Lemmus lemmus*), but as generalists on Collared Lemmings (*Dicrostonyx torquatus*; Angerbjorn *et al.* 1999); in Fennoscandia this canid is a lemming specialist, but opportunistically consumes birds, Reindeer (domestic *Rangifer tarandus*, presumably carcasses), other small mammals, and hares (Elmhagen *et al.* 2000). Red Foxes (*V. vulpes*) are "the archetypical generalist predator" (O'Mahony et al. 1999:575), and consume a broad array of food items across an immense geographical range, as do both Golden (*Canis aureus*; Aleksandra and Dusko 2015) and Black-backed jackals *(Lupulella mesomelas*; Humphries *et al.* 2016). Reflecting this trophic flexibility, the diet of some smaller and mid-sized canids varies regionally (*e. g.*, Etheredge *et al.* 2015).

Two wild canids range over much of the Southern Cone of South America. These are the larger Culpeo Fox (*Lycalopex culpaeus*, 4 to 14 kg; Culpeo hereafter) which ranges along the Andes from Colombia to Tierra del Fuego (excluding wet temperate rainforest; Novaro 1997b), and the smaller and competitively subordinate Chilla Fox (*L. grisea*, 2 to 5 kg; Chilla hereafter), which ranges from southern Perú to southern Patagonia (introduced to Tierra del Fuego—Jaksic and Yáñez 1983; Lucherini 2016). Additionally, the Pampas Fox (*L. gymnocerca*) ranges from eastern Bolivia and western Paraguay south through the Monte, Espinal, and Pampas ecoregions of Argentina (Lucherini and Luengos Vidal 2008), and Darwin's Fox (*L. fulvipes*) has a very restricted range in south-central Chile (Iriarte and Jaksic 2012). The most widely distributed of these, the Culpeo and Chilla, have been studied extensively (reviewed by Jaksic 1998) and both tend to have diverse diets (as does the Pampas Fox; Lucherini and Luengos Vidal 2008). In the coastal fringe of the Atacama Desert, Culpeos consume primarily arthropods, complemented by reptiles, birds, and small mammals (Guzmán-Sandoval *et al.* 2007); Chillas in this area target small mammals but also consume seabirds and crustaceans (Marquet *et al.* 1993). In the southern Andes the Culpeo consumes small mammals (Marquet *et al.* 1993), but this may be complemented by camelids (presumably carrion), European Hares (*Lepus europaeus*), and birds, leading Walker *et al.* (2007) to conclude that Culpeos were more generalist than other mid-sized carnivores in the region (*e. g.*, Andean Mountain Cat [*Leopardus jacobita*], and Colocolo [*L. colocola*]). Where both canid species co-occur in Patagonia, Chillas emphasize arthropods and small mammals in their diet while Culpeos focus on invasive European Hare (Zapata *et al.* 2005; Gantchoff and Belant 2016); Palacios *et al.* (2012) further emphasized the role of invasive species in the diet of Culpeo in this region, arguing that native prey items are ecologically (hence, functionally) extirpated in this region. In steppe habitat of central Argentina, Culpeos appear to retain a preference for native small mammals (by biomass; Pia *et al.* 2003), whereas in mediterranean Chile they appear to have adopted European Rabbits (*Oryctolagus cuniculus*) as their primary prey (Rubio *et al.* 2013), and in forested regions of southern Chile both canids (as well as Darwin's Fox) shifted their apparent prey preferences in exotic plantations (Monterrey Pine, *Pinus radiata*) relative to native forest, selecting arboreal rodents (*Oligoryzomys longicaudatus*, *Irenomys tarsalis*) in the former even though these were more abundant in the latter (Moreira-Arce *et al.* 2015).

This brief overview underscores the adaptability of Culpeo in the face of changing biotic conditions. Efforts to characterize either numerical or functional responses, however, have been limited to two sites in northern Chile. At an interior site in the northern mediterranean region (Las Chinchillas National Reserve), Jaksic *et al.* (1992) and Martínez *et al.* (1993) tracked fox numbers and diet over 45 months and one cycle in prey abundance (mostly native small mammals; invasive lagomorphs had not yet become abundant at this site; F. Jaksic pers. comm. to DAK, October 2016). Jaksic *et al.* (1992:95) reported that Culpeos "consumed highly variable amounts of fruit regardless of the availability of mammalian prey," and while Martínez *et al.* (1993) did not distinguish the scat of Culpeo and Chilla, the combined dietary information showed no indication of a functional response; rather, foxes exhibited strong preference for Common Degus (*Octodon degus*; Degu hereafter) and Darwin's Leaf-eared Mice (*Phyllotis darwini*). At a coastal site *ca*. 90 km from Las Chinchillas (Bosque Fray Jorge National Park; Fray Jorge hereafter), Jaksic *et al.* (1993, 1997) reported somewhat similar dynamics; Culpeos (the only fox present at that site) were selective predators, favoring Bennett's Chinchilla Rat (*Abrocoma bennettii*) and Degus, but avoiding *P. darwini*, although they increased consumption of fruits when small mammal num-

bers were low (Castro *et al.* 1994). Finally, whereas Jaksic *et al.* (1993) reported no numerical responses across variation in prey abundances, Jaksic *et al.* (1997) suggested a modest numerical response, although this was confounded by the fact that these authors combined multiple predator species (Culpeo and raptors) in their assessment of numerical responses. Neither study at Fray Jorge (Jaksic *et al.* 1993, 1997) suggested functional responses by Culpeos, which continued to emphasize small mammals throughout the study period, albeit complemented occasionally by arthropods and fruit/seed.

One potential complication in a region known for extensive temporal variation in abundance of prey species (Iriarte *et al.* 1989a; Jiménez *et al.* 1992; Meserve *et al.* 2016) is that all studies on the diet of the Culpeo have been limited in the temporal extent of observation. We have been studying the ecology of small mammals at Fray Jorge since 1989, including monthly assessments of population sizes at a series of replicate study plots (reviewed in Meserve *et al.* 2003, 2016; Gutiérrez *et al.* 2010) and complemented with monthly collection of Culpeo scat along standardized routes. This is the same site reported on by Jaksic *et al.* (1993, 1997), but here we report on a much longer (23-year) dataset spanning extensive temporal variation in small mammal numbers, and simultaneous variation in Culpeo diets.

Two events at this site prompted this updated analysis. First, climatic patterns appear to have shifted in about 2000/2002. Prior to this, the site experienced periodic El Niño Southern Oscillation wet periods every 3 to 5 years, with strong numerical responses by small mammals (Meserve *et al.* 1993, 1996). After about 2002, annual rainfall declined and became less variable (Meserve *et al.* 2011, 2016; Armas *et al.* 2016a); subsequent to this climatic change, we documented a major shift in the composition of the small mammal fauna, the most notable change being a tremendous proportional increase in Degu abundance. Since 2001 this species has comprised at least 50 % of the biomass of small mammals at our site, and over 65 % since 2010 (Meserve *et al.* 2016). If Degus are a favored prey of Culpeo (Jaksic *et al.* 1993, 1997), then we would predict that this predator would respond to this change in prey availability.

The second major change at the site is that invasive lagomorph populations have increased greatly, starting in late 2002 or early 2003. This appears to be a response to two unrelated events. The first of these was that 2000 to 2002 comprised a series of three years of elevated rainfall at our site, leading to abundant food for herbivorous or folivorous consumers. Additionally, Culpeos at Fray Jorge experienced a distemper outbreak in 2002/03 (Moreira and Stutzin 2005; Acosta Jamett 2010) which led to a substantial decline in fox numbers and presumably in predation pressure on lagomorphs. In our field notes from that time period we document encountering numerous fox carcasses, and for the first time noting both European Hares and European Rabbits commonly. Hares and rabbits have remained abundant throughout the park since this time (DAK, pers. obs.).

Given the existing literature on the population ecology of Culpeos, we predicted that they would respond numerically but not functionally to changes in prey abundance. We predicted that Culpeos would favor some species as prey (*e. g.*, larger small mammals but also lagomorphs once the latter became abundant at our site), but that they would show no sign of adjusting their foraging to changes in prey availability.

Materials and methods

Study site. Since 1989 we have maintained a long-term study on the ecology of arid lands in Fray Jorge, at the northern fringe of mediterranean Chile (reviewed in Squeo *et al.* 2004; Armas *et al.* 2016b). Initially established to assess the relative importance of biotic influences (competition and predation) on small mammals (Meserve *et al.* 1993) and top-down impacts on plants (Gutiérrez *et al.* 1997), we have refocused effort to target the relative importance of biotic versus abiotic drivers after episodic rainfall, generally associated with the El Niño Southern Oscillation (ENSO) phenomenon. Such events effectively "reset the clock" of predator-prey dynamics, with rainfall promoting primary productivity and providing abundant food for small mammals, effectively releasing them from any top-down control by predators (Meserve *et al.* 2003, 2016). After multiple wet/dry cycles we have documented predictable responses by most biotic elements, and research emphasis has shifted to monitoring diverse biotic and abiotic parameters in the face of the apparent "regime change" (*sensu* Scheffer 2009) in 2000/02.

The main part of Fray Jorge comprises about 9,000 ha on the coast of north-central Chile, located approximately 400 km North of Santiago and 150 km South of the southern border of the Atacama Desert. The region is semi-arid but receives a strong oceanic influence. Coastal hills (the Altos de Talinay, *ca*. 640 m elevation) intercept fog and support remnant patches of forest with numerous elements of Valdivian temperate rainforest, characteristic of southern Chile (Villagrán *et al.* 2004; Squeo *et al.* 2016). Our study is in the Quebrada de las Vacas, a north-south oriented valley located just interior to the Altos de Talinay, and dominated by spiny drought-deciduous and evergreen shrubs with a seasonal ephemeral plant understory (Gutiérrez *et al.* 1993; Gutiérrez *et al.* 2004; Squeo *et al.* 2016). The climate is semi-arid Mediterranean with warm summers and cool winters. Mean annual precipitation is just under 127 mm, most of which falls in winter (May to October).

The small mammal community of Chile is well known (Osgood 1943; Jaksic 1998; Muñoz-Pedreros and Yáñez 2009), and the fauna of Fray Jorge has been well studied since the 1970s (Schamberger and Fulk 1974; Fulk 1975; Meserve 1981a, b: Meserve *et al.* 1993, 1995, 1996, 2001, 2003, 2016; Yunger *et al.* 2002; Kelt *et al.* 2004a, b, c). The community is diverse and includes three herbivorous caviomorph rodents (*Octodon degus* [*ca*. 120 to 180 g], *O. lunatus* [*ca*. 160 to 200 g], and *Abrocoma bennettii* [150 to 250 g])

and several smaller (20 to 80 g) sigmodontine rodents. The most abundant sigmodontines are the omnivorous *Abrothrix olivacea* and omnivorous / herbivorous *Phyllotis darwini*, but lesser numbers of insectivorous *A. longipilis* and granivorous *Oligoryzomys longicaudatus* are recorded as well (dietary characterization from Meserve 1981a). Finally, a carnivorous / frugivorous mouse opossum (*Thylamys elegans*, 25 to 35 g) occurs here and is regularly captured. We classify species as "core", "quasi-core", or "opportunistic", based on their apparent dependence on thorn scrub habitat, where our sampling is based. *Octodon degus*, *P. darwini*, and *A. olivacea* are captured every month and are considered to be core species. *Thylamys* is captured in most months but with less consistency, and we frequently treat this as a quasi-core species. All of these species may also be found in other key habitats at Fray Jorge, including fog forest that occurs on the adjacent Altos de Talinay and in moister aguadas habitat, where the water table is sufficiently high to support more mesic vegetation. Supplemental trapping in these habitats (Milstead *et al.* 2007) suggests that *A. longipilis* is primarily a species of the fog forest, but it enters thorn scrub habitat when conditions facilitate population expansion, such as during rainy periods. *Oligoryzomys* also appears to favor moister habitats (both fog forest and aguadas), as does *O. lunatus*. Because these species may disappear from our thorn-scrub trapping grids for months at a time, we consider these to be opportunistic residents. Further information on these species and the broader structure of our research program may be found in Armas *et al.* (2016b).

Small mammal abundances. Small mammals have been surveyed monthly on 16 to 20 replicate plots allocated to 4 to 6 experimental treatments that include selective exclusion of predators or subsets of the small mammal assemblage (Kelt *et al.* 2013; Meserve *et al.* 2016). For the purposes of this study we only use data from four control plots that have not been subjected to any biological exclusion through the duration of the study. All plots are 75 x 75 m in area, and control plots are encircled with a low fence (*ca*. 0.6 m) using "chicken-wire" (2.5 cm hexagonal mesh) to which we facilitate access by all small mammals by cutting 5 cm dia. holes at 5-m intervals (Meserve *et al.* 1993). Each plot includes a 5 x 5 trapping grid, with 15-m spacing between stations. Monthly surveys comprise four consecutive nights and days using 50 large Sherman-type live traps (10.5 x 11.3 x 30.5 cm; two traps per station). All captured animals are identified to species and uniquely marked with numbered eartags or leg bands. All field efforts meet criteria established by the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016) and have been approved by Institutional Animal Care and Use Committees at our respective institutions.

We used the superpopulation model (Schwarz and Arnason 1996; Williams *et al.* 2002) to estimate population size for small mammal species occurring on our control grids (n = 4) from December 1990 through August 2013. To match seasonal dynamics at Fray Jorge we calculated mean values over 3-month periods (Summer, Dec to Feb; Fall, Mar to May; Winter, Jun to Aug; Spring, Sep to Nov.), although in some presentations we employ 6-month periods (reproductive, Sep to Feb; non-reproductive, Mar to Aug; hence, reproductive season "R1990" would span September 1990 through February 1991). Demographic modeling was conducted in Program MARK (White and Burnham 1999) using the RMark package (Laake 2013) for the R computing environment (R Core Team 2024), as detailed in Kelt *et al.* (in prep).

Numerical responses by Culpeo. To assess Culpeo activity at our site we established three olfactory lines (21 scent stations each, approximately 100-m intervals) among the small mammal trapping plots (Previtali *et al.* 2009). Each station consisted of a *ca*. 1 m dia. plot of sifted and smoothed sand that was cleared of vegetation. We surveyed for fox activity ≥2 days per month by placing a cotton-wrapped stick soaked in predator lure (Bobcat #1 lure; Cronk's Outdoor Supplies, Wiscasset, Maine, USA) in the center of each station. Following Previtali *et al.* (2009) we estimated fox activity as the number of scent stations visited (based on scratch marks, tracks, removed lure, etc.) during the first two days of olfactory surveys. Data were tabulated in 3-month windows for comparison with small mammal population estimates.

Culpeo diet. Culpeo scat have been collected monthly at our site since March 1989. Scat are collected in the Quebrada de las Vacas where all of our small mammal censuses were conducted. As noted by Jaksic *et al.* (1992), deterioration over a single month is trivial in this arid region, and sampling on established routes ensured that sampling intensity remained approximately equal in all months. After drying, scat were physically separated and all items identified by trained technicians, using a dissecting microscope as needed. For animal prey other than lagomorphs, we determined the minimum number of individuals of each taxon on the basis of identifiable structures (*e. g.*, mandibles, crania, antennae, wings, etc.). Most lagomorph remains consisted of bone fragments and occasional teeth, effectively precluding estimation of the number of individuals in a given scat.

We tested for seasonal differences in diet (reproductive vs. non-reproductive) for basic dietary groups (mammals, birds, etc.) using *t*-tests. The only groups suggesting seasonal differences were ectothermic (reptiles) or partially migratory (birds); because these are minor elements of the diet of Culpeos (representing, on average, 6 and 5 %, respectively, of prey remains in seasonal surveys) and we lack estimates of actual densities for comparison against frequency of consumption, we omit these groups from further consideration. Because small mammals dominate the diet (see Results) and because we have contemporaneous estimates of population density, we restrict our attention to these prey items in most of the following analyses.

We calculated basic descriptive parameters for each seasonal diet sample. These included proportion of items comprising key species, number of scat sampled, and three metrics of diet composition – diet breadth, diet diversity, and diet evenness. Following Jaksic *et al.* (1992, 1993) diet breadth was calculated as $B = 1/\sum p_i^2$ where p_i is the proportional representation of prey item *i* in the diet during a given time period. With *n* diet categories, *B* ranges between 1 and *n*, and provides an index of resource use that is independent of relative availability (Feinsinger *et al.* 1981). We calculated diversity with the Shannon diversity index ($H' = \sum p_i [ln(p_i)]$ which ranges from 0 to $ln(S)$ where *S* = the number of species present (Magurran 1988). Finally, evenness was calculated as $E = H'/\ln(S)$. *E* ranges from 0 to 1, with higher values reflecting increased numerical similarity across categories (Magurran 1988). We applied these metrics at two scales of analysis, first assessing general patterns of consumption across broad trophic categories (*e. g.*, mammal, bird, etc.) and subsequently assessing consumption by different species of small mammals (both with and without invasive lagomorphs, see below).

We characterize prey species as "selected" when their frequency in scat samples (see Supplementary material Table S2) exceeded their relative abundance based on live trapping, and as "avoided" or "negatively selected" when the frequency in scat was less than their relative abundance based on live trapping (see Supplementary material Table S3). We conservatively tested for significant deviation from random variation with a binomial test of the number of periods in which ratios of selection or avoidance were ≥1.5.

Non-native lagomorphs. European Hares and European Rabbits have been present in mediterranean Chile for decades (Camus *et al.* 2008; Jaksic and Castro 2014), but neither was markedly abundant at Fray Jorge until about 2003, following three sequential years of above-normal rainfall and a distemper outbreak that greatly reduced the Culpeo population (Moreira and Stutzin 2005; Acosta Jamett 2010). Once Culpeo numbers declined, both species of lagomorphs became notably abundant, but this was particularly true for *Oryctolagus*. Fox numbers rebounded in subsequent years (DAK, pers. obs.), but because of the increase in lagomorph remains in fox scat in several years (*e. g.*, 2003 to '07, 2011) we calculate diet breadth and diversity both with and without lagomorphs.

Lagomorphs typically are surveyed with live-trapping or distance sampling methods (*e. g.*, Palomares 2001). Unfortunately, European Hares and European Rabbits are not readily live-trapped, and extensive efforts with distance methods have proven fruitless at this site, where high shrub cover (>50 %) limits visibility, and even nocturnal spotlighting efforts yield very few individuals. Therefore, in 2008 we established a series of 54 pellet-count stations (cf. Murray *et al.* 2002) to obtain standard indices of lagomorph numbers. Each station consists of a 1-m circular area cleared of vegetation and existing pellets. We placed a stake at the center of each station and at *ca*. 6-mo intervals (generally August and February/March) we counted and removed all pellets present. This interval conformed to the timing of site visits by the principal investigators. Although pellets of adult *Lepus* are readily distinguished from those of adult *Oryctolagus*, variation in pellet size reflecting the size and age of the animal precluded separate metrics for each taxon; instead, we present a single metric of lagomorph activity, although field observations make it clear that *Oryctolagus* is much more abundant than *Lepus*.

In 2011 we established a second effort to survey lagomorphs, consisting of 21 stations at *ca*. 500-m intervals along the only dirt road in our study site. At each station we cleared vegetation and sifted fine soil over two *ca*. 1.5-m dia. circular plots located 3 to 5 m apart. At the center of each plot we placed a single Petri dish filled with approximately 2 to 5 mm plaster of Paris. Upon placement, we added about 4 to 5 drops of scent, consisting of either mint or carrot extract (one of each at every station) dissolved in propylene glycol to inhibit evaporation. Stations were smoothed with a fine brush, and dishes placed in the evening, and checked early in the morning for tracks or pellets. We surveyed stations for four consecutive nights, replacing scent each evening. With 8 ½ years of data for both survey methods, total pellet counts (excluding the extreme high value from each season) and olfactory plot visits exhibit very high correlation ($F_{1,15} = 12.15$, $P = 0.0033$), although there remains noise in these data (adjusted $R^2 = 0.41$). While neither metric can be tied to actual lagomorph numbers, the agreement between these independent assays suggests that they provide a useful index of lagomorph abundance.

Functional and numerical response. We follow Jaksic *et al.* (1996, 1997) in assessing numerical responses by Culpeos to variation in small mammal abundance by regressing the abundance of foxes (as inferred from olfactory lines; Previtali *et al.* 2009) against small mammal population density (on control plots). Theory predicts a counter-clockwise trajectory of data in such a bivariate plot; as small mammal numbers (x axis) increase, at some point consumption by foxes (y axis) should increase, and this pattern should reverse as small mammal numbers decline. Such patterns frequently are challenging to quantify, however, given large interannual variation in prey abundances.

We assessed functional responses to variation in small mammal prey items in two ways. First, we characterized patterns of prey consumption by comparing consumption of different small mammal species and associated dietary metrics (breadth, diversity, evenness) as functions both of sample sizes (number of scat analyzed) and of prey population size. Second, we plotted our best estimate of per capita consumption (*e. g.*, the mean minimum number of prey items per scat) against population size across 45 6-month sampling periods. We characterized Type I, II, and III functional responses with linear, quadratic, and sigmoidal non-linear regression, respectively, which we compared against a null model corresponding to a constant value of the mean number of prey items consumed across

all prey densities. We compared regression models with Akaike's Information Criterion corrected for small sample size (AICc), and considered models to be competitive if they had Akaike differences (ΔAICc) ≤ 2.0 and relatively high Akaike weights (w_i).

Results

Temporal patterns in small mammal numbers and Culpeo activity. Small mammal populations have fluctuated greatly over time and generally are strongly influenced by rainfall (Meserve *et al.* 2016; Figure 1). The greatest numerical changes were documented for *Abrothrix olivacea*, *Octodon degus*, and *Phyllotis darwini*, with lesser changes by most other species. Culpeo activity at our site, as measured by the number of olfactory stations visited, varied over the duration of our study, and generally increased during and after rainy periods (Figure 1), but in general these data suggest that fox activity fluctuated substantially around a long-term mean (*ca*. 170 station visits monthly) through the study period.

Culpeo dietary selectivity. We analyzed 4,769 scats collected over 23 years (Supplementary material Figure S1, Table S1). These contained ≥8,297 individual animal prey items, 5,128 (61.8 %) of which were mammals, 468 (5.6 %) birds, 373 (4.5 %) reptiles, and 2,328 (28.1 %) invertebrates. It is clear, however, that mammalian prey were both the dominant and most consistent food consumed; arthropods comprised significant components of the diet only in about 12 of 46 6-month periods (Figure 2a), and their biomass contributions were substantially less than that of small mammals. We also tallied 261,703 seeds (Figure 2b), supporting earlier observations that Culpeo consume considerable fruit (Jaksic *et al.* 1992; Castro *et al.* 1994), although these appear to be somewhat sporadic in occurrence in scats (Figure 2b).

Because we have data on both use (consumption) and availability (control grids) of small mammals we are able to assess selectivity of these prey by foxes. The proportional representation of seven species of small mammals was relatively invariant across both the number of scat collected (one metric of fox activity; Figure 2c) and total small mammal abundance (an index of prey availability; Figure 2e), although these data indicate clearly that *Abrocoma* and *Octodon* comprise the majority of mammalian food items consumed by Culpeos (Figure 2c, 2e). Three dietary metrics (breadth, diversity, evenness) exhibited trivial variation with respect to the number of scat sampled (Figure 2d), and while all three varied significantly as a function of small mammal population size (Figure 2f), they explained less than one-third of the variance in the available data. Perhaps most notably, Culpeo diet breadth regressed positively on small mammal population size; Culpeos tended to eat fewer species when prey populations were low, and more species when these populations were high. The relative contribution of lagomorphs is more difficult to assess, because we lack estimates of their abundance for most of the study period, and the number of items in scat do not necessarily represent separate individuals. However, Figure 2a suggests that lagomorphs have become more common in Culpeo diets since about 2003.

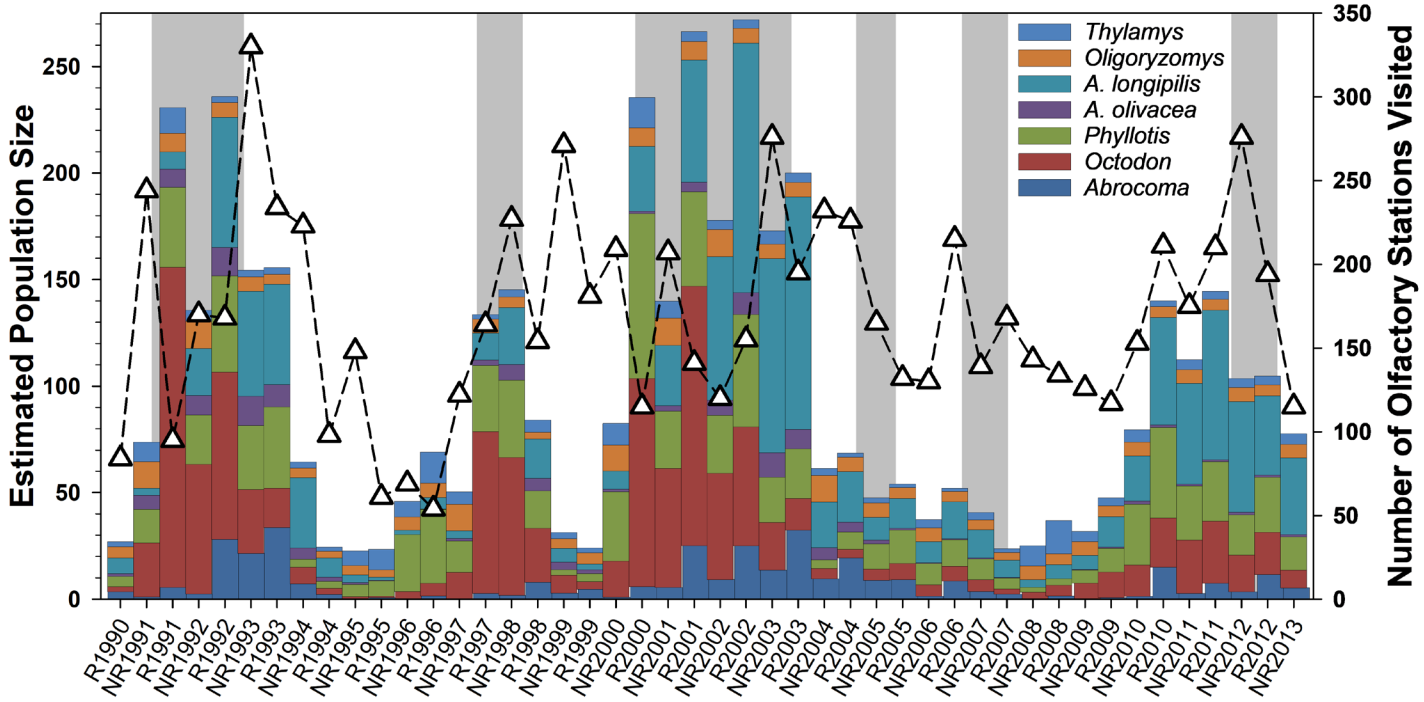

Figure 1. Estimated population sizes for small mammals on four control trapping grids over 23 years at Fray Jorge. Triangles and dashed lines indicate the number of olfactory stations visited by Culpeo (*Lycalopex culpaeus*) in 6-mo periods from December 1990 through August 2008 (except the first bar which represents only three months of observation (Dec. to Feb.), and NR1994 which is missing data for June 1994). NR = period of non-reproductive activity (*e. g.*, NR1991 = Mar. to Aug. 1991); R = period of small mammal reproduction (*e. g.*, R1991 = Sep. 1991 to Feb. 1992). Periods of high rainfall are indicated with gray shading.

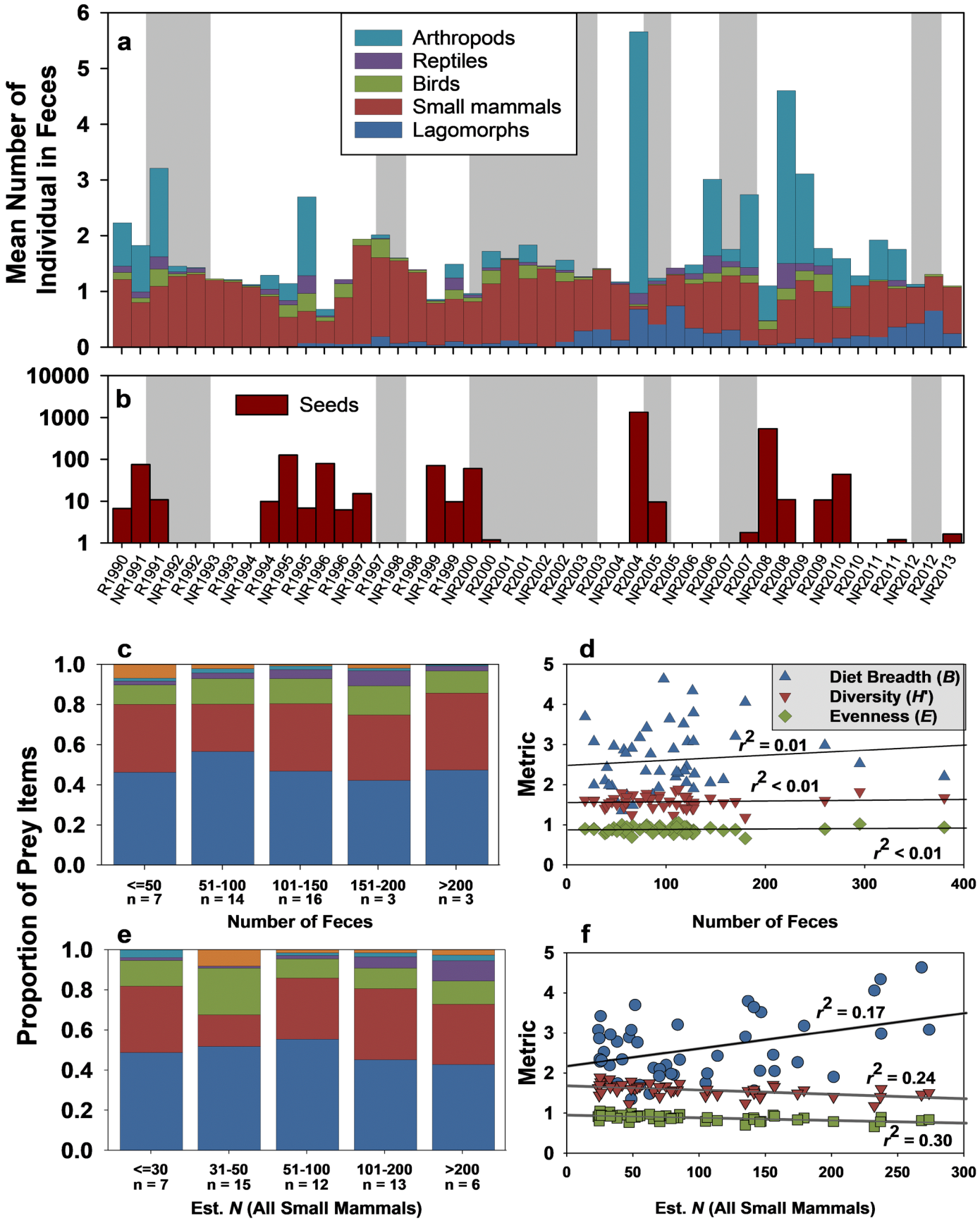

Figure 2. General dietary composition for Culpeo (*Lycalopex culpaeus*) across 23 years at Fray Jorge, Chile. (a) Seeds are present in the diet sporadically and in highly variable numbers (note the log axis). (b) Culpeo emphasize small mammals in their diet even as small mammal numbers vary greatly (cf. Figure 1). Lagomorphs have increased in representation since about 2002. Other taxa are consumed more opportunistically over time. The proportional composition of small mammal remains in Culpeo scat is invariant across (c, d) variation in number of scat sampled (as one metric of fox abundance or activity) or (e, f) the number of individual mammals in scat (as an index of small mammal population sizes). Panels d and f present metrics pertaining to panels c and e, respectively; linear regressions in d are non-significant (all $P > 0.45$), those in f are all significant (all $P < 0.005$). Panels c through f do not include lagomorphs.

Seasonal analysis of Culpeo diet provides greater resolution of prey selectivity (Figure 3). Most species of small mammals were consumed in greater proportion to their availability during some seasons and in lesser proportions in others. The only species consistently over-represented was the large herbivorous *Abrocoma*; this species was positively selected in 44 of 46 6-month seasons (Figure 3a, b). In contrast, *A. olivacea*, *Oligoryzomys*, *Phyllotis*, and *Thylamys* all were better represented in our monthly surveys than they were in Culpeo scat, therefore being selected against in most seasons, and selected for in just zero or one season each. In contrast, both *O. degus* and *A. longipilis* were selected for and against at roughly similar rates (11 and 13 seasons for the former, seven and five for the latter).

For those species that were positively selected, selectivity was greatest when those species were low in abundance (Figure 3, green symbols in right panels). There was some indication of similar patterns for avoidance (*e. g.*, *Octodon* and *Phyllotis*, for example; red symbols) but this was less consistent than the pattern for positive selection.

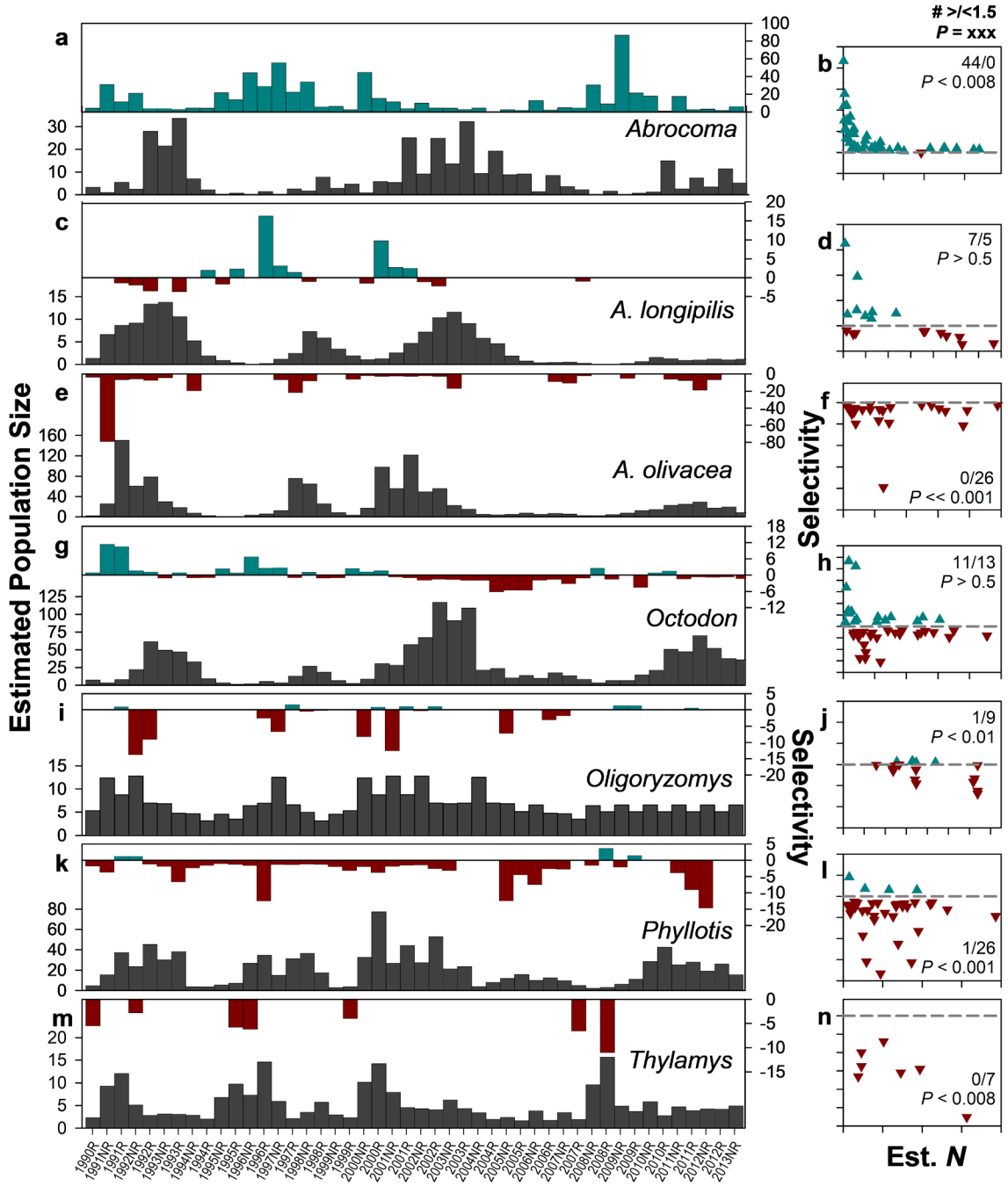

Figure 3. Selectivity of seven species of small mammals in the diet of Culpeo (*Lycalopex culpaeus*) across 23 years at Fray Jorge, Chile. Black vertical bars present population estimates calculated over 6-mo periods, while green and red vertical bars represent selection and avoidance, respectively, of these species in the same periods. Scatterplots to the right show selectivities as a function of population estimates for each small mammal species; dashed gray line reflects zero selectivity, and values presented are the number of positive and negative selectivity values >1.5 and associated probabilities in a binomial test (see Supplementary material Table S4).

Numerical responses. Using all data points (n = 101 3-month periods; Figure 4a) fox activity is only modestly associated with small mammal abundances (linear regression, P = 0.10) and the relationship explains little variation (R^2 = 0.026) and has a very low slope (β = 0.021). However, of interest here is whether these data tend to comprise counter-clockwise cycles, with predators responding positively to higher small mammal populations, and negatively to lower populations. When these data are partitioned into sequential multi-year cycles the results are suggestive, although temporally variable as one might expect given interannual heterogeneity at this site (*e. g.*, rainy versus dry years). However, in most cycles the data generally follow the expected counter-clockwise trajectory (Figures 4b-g). Years when this pattern is less clear (*e. g.*, 2004 to 09, 2009 to 13) are relatively dry years in which small mammal abundances remained relatively low; under such conditions one would not expect substantial demographic response by Culpeos, and this was observed.

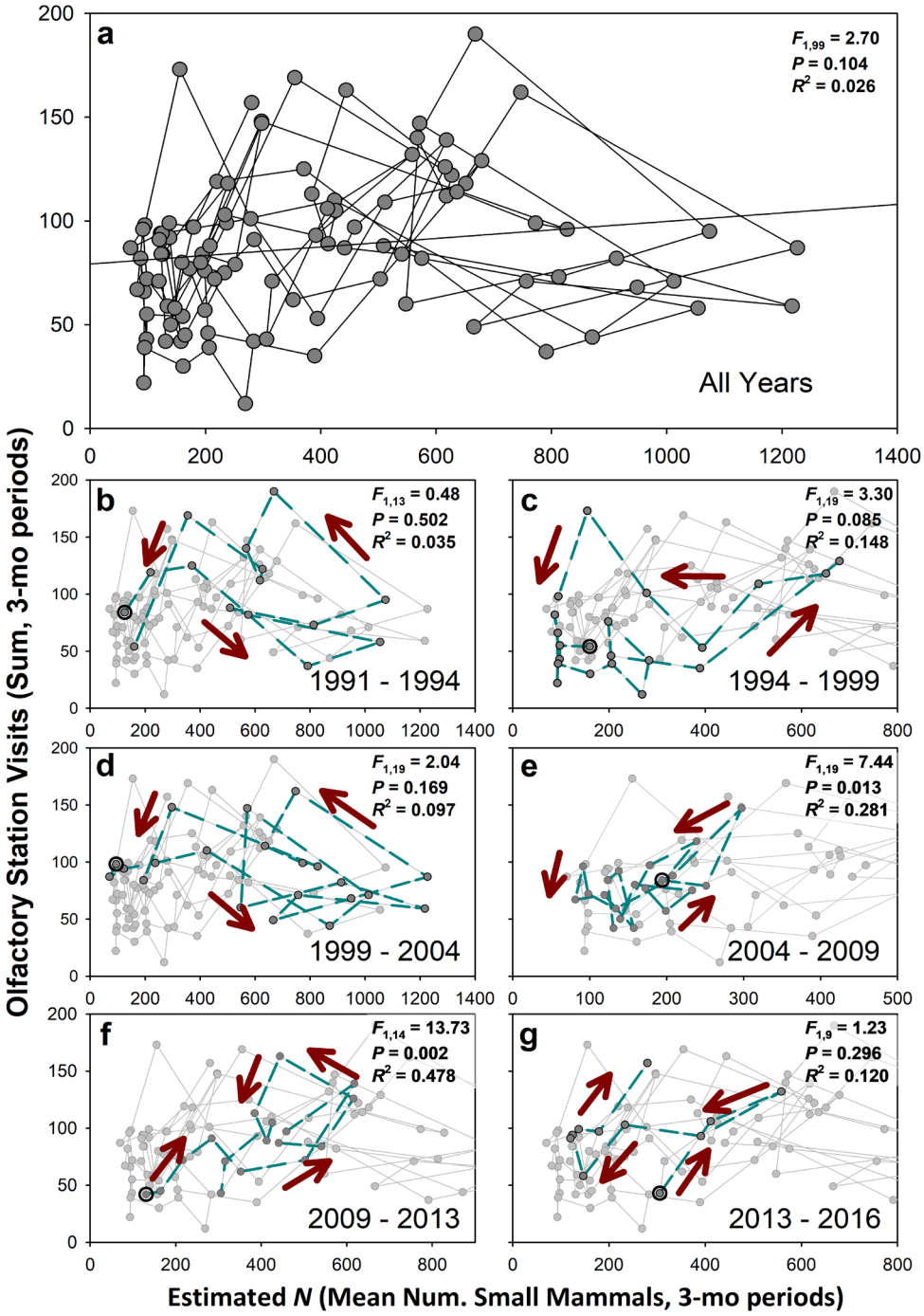

Figure 4. Numerical response of Culpeo (*Lycalopex culpaeus*) to temporal variation in small mammal populations across 23 years at Fray Jorge, Chile. The first panel presents all time periods (101 3-mo periods), while subsequent panels highlight separate multi-year cycles suggestive of prey tracking by Culpeo (*e. g.*, variably counter-clockwise trajectories). Note that the x-axes in panels e through g have been adjusted to illustrate patterns during cycles with relatively low small mammal population. Red arrows illustrate the trajectory of data points (large circle indicates starting point for each panel). A similar analysis with 6-mo windows increases the fit to the data (linear regression, $F_{1,44}$ = 10.62, P = 0.0022) but the explanatory power remains low (R^2 = 0.19).

Functional responses. Whereas proportional consumption of broad trophic categories as well as different small mammal species in scat samples is effectively invariant across both Culpeo and small mammal abundance (Figure 2), per capita consumption of small mammals clearly increases with availability (Figure 5). This observation rejects our hypothesis of no functional response, leaving open the question of the nature of the functional response (*e. g.*, Type I, II, or III). Perhaps surprisingly, the available data are not explained better by any of these models; AICc values are not greatly different, such that Akaike weights (w_i) decline from 0.39 to 0.28 across these three models, and evidence ratios reaffirm the lack of a single dominant model (Table 1). Similarly, the variation explained by these models ranges from 20 % (Type I) to 25% (Type III).

Table 1. Regression of consumption of small mammals by Culpeo (*Lycalopex culpaeus*) against estimated population size across all small mammals at Fray Jorge, Chile. Although we assume that foxes satiate (stomach size is finite so consumption should asymptote) we applied linear, polynomial, and sigmoidal regressions as estimates of Type I, Type II, and Type III functional responses, respectively. A null (intercept) model was employed for comparison against a lack of any functional response. All models were compared using Akaike's Information Criterion (AICc) and Akaike weights (w_i).

Order	Model	R^2	AICc	ΔAICc	w_i
Type I	$y = 0.517 + 2.07 \times 10^{-3} (x)$	0.202	-106.637	0	0.389
Type II	$y = 0.379 + 5.3 \times 10^{-3} (x) - 1.24 \times 10^{-5} (x^2)$	0.237	-106.296	0.341	0.328
Type III	$y = 0.99/(1 + e^{-(x-62.17)/20.73})$	0.247	-105.997	0.641	0.283
Null	$y = 0.723$		-52.570	54.067	7×10^{-13}

We reject a Type I functional response, both because the data suggest a clear threshold in per capita consumption (Figure 5) and because handling time, stomach capacity, and digestive physiology all would be expected to limit consumption at some level of prey availability. Our data appear insufficient to distinguish between a Type II and Type III model, although Occam's Razor would encourage acceptance of the less complex Type II model. As such, we interpret these data to suggest a weak Type II functional response with an apparent asymptote above a population density of about 100 individuals (Figure 5).

Influence of non-native lagomorphs. Non-native lagomorphs were consumed by Culpeos throughout our study, but their representation in scat increased modestly in 1997 and then markedly in 2003 (Figure 6). Consumption subsequently declined but then increased again after mid-2011. Overall, however, relative to small mammals, lagomorphs remain minor diet elements, and summary statistics (diet breadth, *H'*, evenness) do not vary notably when lagomorphs are incorporated (data not shown). Although data remain limited, a regression of the abundance of lagomorphs in Culpeo scat on five years of lagomorph pellet count data (an estimate of lagomorph abundance) are suggestive of a positive association, but this remains nonsignificant ($F_{1,9} = 3.24$, $P = 0.11$, $R^2 = 0.26$; Figure 6).

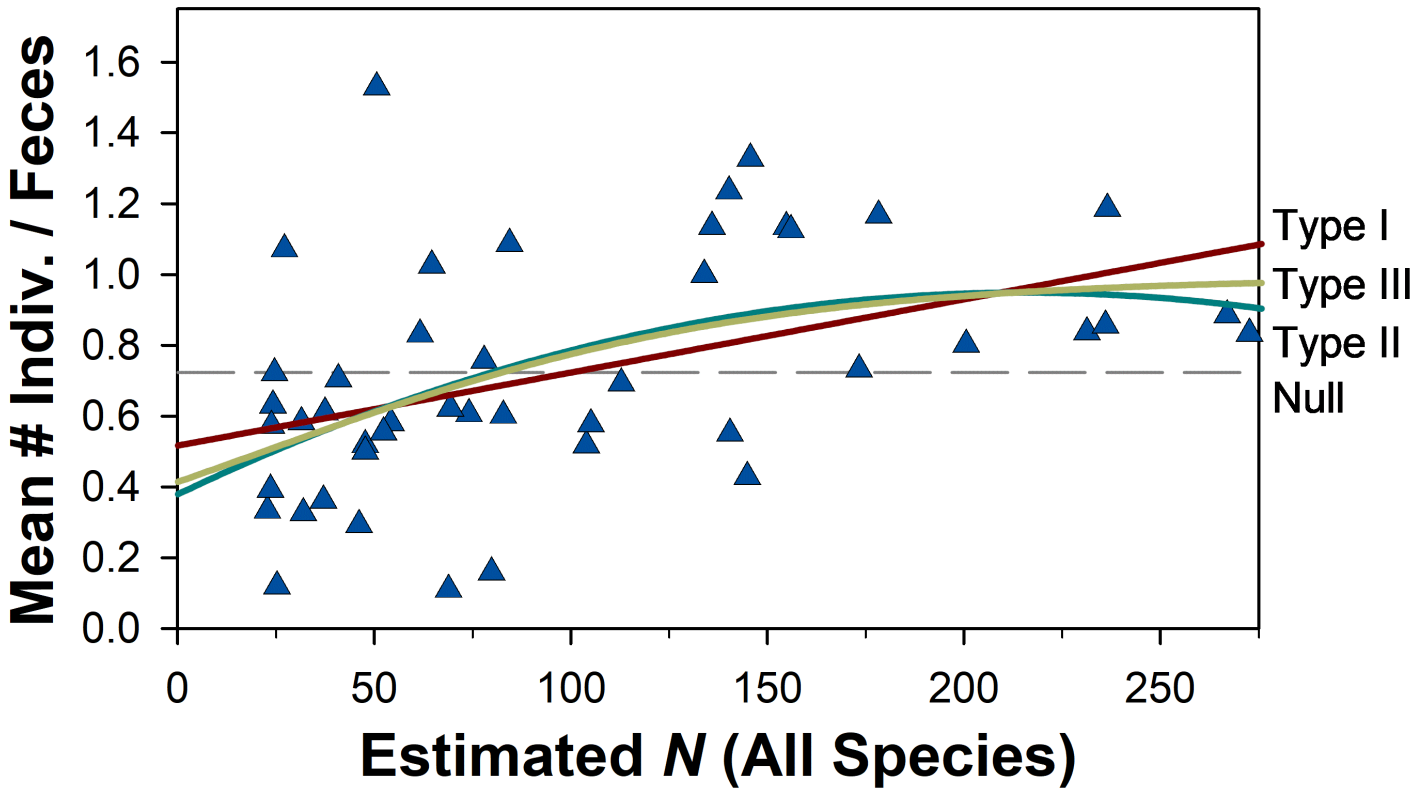

Figure 5. Per capita consumption of small mammals (mean number of individuals per scat) by Culpeo (*Lycalopex culpaeus*) as a function of estimated population size in 6-month periods over 23 years at Fray Jorge, Chile. Type I, II, and III regressions provide similar representation of these data (ΔAICc = 0.34 and 0.64 for the latter two models; see Table 1 for regression results). The principle of parsimony would argue for the simpler model, whereas foraging ecology (*e. g.*, handling time, finite stomach and digestive capacity) suggests that foxes must satiate at some level of prey density; in combination, these arguments suggest that a Type II functional response best expresses Culpeo foraging on small mammals.

Discussion

Culpeos are facultative specialists. In the face of extensive variation in availability of prey, Culpeos maintain a diverse diet, but small mammals remained the foundation of this diet over the 23-years of this study. Moreover, Culpeos are highly selective, consuming *Abrocoma bennettii* disproportionately in all months of study, and varying in the extent of preference for, or avoidance of, *Octodon degus*, relative to their contribution to local assemblages. Reflecting earlier work at this site, Culpeo appear to avoid *Phyllotis*, but unlike earlier studies they also avoided *A. olivacea* and the sole marsupial at our site, *Thylamys*. *Phyllotis* and *Thylamys* are largely nocturnal, and *A. olivacea* ranges from nocturnal to crepuscular to even diurnal, likely as a function of abundance and associated resource availability. Culpeos are labile foragers and are capable of foraging at any hour of the day, evidently as a function of the availability of favored prey (Iriarte *et al.* 1989b; Johnson and Franklin 1994) as well as human persecution. In Argentina, highland Peru, the Chilean desert and Magallanes, Culpeos are almost completely nocturnal (Crespo and de Carlo 1963; Crespo 1975; Johnson 1992; Novaro 1997a, b). This contrasts with their largely diurnal activity patterns in north central Chile (Jiménez 1993; Salvatori *et al.* 1999), where Culpeos are protected. Hence the apparent avoidance of *Phyllotis*, *A. olivacea*, and *Thylamys* may reflect the largely nocturnal habits of these species at Fray Jorge. Additionally, *Thylamys* is an insectivorous / frugivorous marsupial and may simply be unpalatable; they have a distinct odor (frequently notable to field workers while animals are still in a closed trap) and when handled they often defecate very moist and somewhat noxious scat. Finally, it is worth noting that all three of these species are relatively small and would provide limited resources on an individual basis.

Avoidance of nocturnal species, though, contrasts with the clear preference for *Abrocoma*, which is generally nocturnal in our study area. However, this species also is the largest rodent in our study area, and while relatively uncommon on our study grids, our data on availability of *Abrocoma* likely reflect underestimates as this species appears to be more abundant in more mesic aguada habitats located near the study plots (Milstead *et al.* 2007, DAK pers. obs.). Further work is needed to better characterize both the spatial patterns of abundance of this species, and its temporal patterns of activity.

We do not fully understand the mixed responses to Degus, which are diurnal, highly social, relatively abundant at Fray Jorge, and among the larger prey items available there. Degus generally are thought to be a favored prey of Culpeos (Meserve *et al.* 1987; Iriarte *et al.* 1989b), and this species is the second most common small mammal in Culpeo scat (Figure 2c, e). At our study site, however, we found no evidence that Culpeos preferentially fed on Degus more than expected by random chance, with no apparent relation to prey abundance except at the very highest densities (Figure 3h).

Neither *Oligoryzomys* nor *A. longipilis* appear to be important elements of Culpeo diets at Fray Jorge, and neither species was selected or avoided by Culpeos in our study; as noted earlier, however, both of these species are more characteristic of other habitats at Fray Jorge (Milstead *et al.* 2007; Meserve *et al.* 2016), and so our estimates of availability may be biased.

As a generality, Culpeos are opportunistic and solitary predators, but they exhibit a highly variable diet, both spatially and temporally (Jiménez and Novaro 2004; Guntiñas *et al.* 2017; Guntiñas *et al.* 2021; Lozano *et al.* 2024). Principal prey range from wild ungulates to domestic sheep, native and introduced lagomorphs, and small mammals; generally, other vertebrates (lizards, birds) and insects make up a small component of Culpeo diet, although insects may be locally or seasonally important (Correa and Roa 2005). In Argentine Patagonia, Culpeos selected among rodent species for those that may be more vulnerable (Corley *et al.* 1995). Culpeos in central Chile select the largest small mammals available (Meserve *et al.* 1987; Iriarte *et al.* 1989b; Jaksic *et al.* 1993). Culpeos have been considered trophically restricted (*e. g.*, strict carnivores, near-insectivore, or generally frugivorous—Iriarte *et al.* 1989b; Cornejo and Jimenez 2001; Guzmán-Sandoval *et al.* 2007, respectively), or as a highly plastic predator capable of capitalizing on a broad range of resources (Jaksic *et al.* 1993; Castro *et al.* 1994; Johnson and Franklin 1994). Integrating across studies, Guntiñas et al. (2017; see also Guntiñas *et al.* 2021; Lozano *et al.* 2024) have argued that Culpeos are facultative specialists, presumably with local preferences reflecting optimal foraging strategies. Ours are the first data to support such a characterization over an extended period.

These foraging characteristics resemble those of many other medium-sized canids. Coyotes (*Canis latrans*) are opportunistic, generalist predators that eat a variety of food items, typically consuming items in relation to changes in availability. Coyotes may adjust activity patterns seasonally or in response to human disturbance or persecution (Kitchen *et al.* 2000), as observed for Culpeos. Similarly, Black-backed and Side-striped jackals (*Lupulella adusta*) are opportunistic and omnivorous predators and scavengers whose diet varies according to food availability (Atkinson *et al.* 2002; Loveridge and Macdonald 2002, 2003; Sillero-Zubiri *et al.* 2004; Skinner and Chimimba 2005; Sillero-Zubiri 2009). Red Fox (*Vulpes vulpes*) are adaptable and opportunistic omnivores, with a diet ranging from invertebrates (*e. g.*, earthworms and beetles) to mammals and birds (including game birds), and fruit. They also scavenge in rural areas. Also similar to Culpeos, Red Fox forage mainly during nocturnal and crepuscular periods, although they are more diurnal where undisturbed (Macdonald and Reynolds 2004).

Culpeo foxes exhibit both numerical and functional responses. Culpeo foxes occur in a wide variety of habitats over a large geographic range (see Introduction). Such a large range would suggest trophic flexibility, and as out-

lined above, this species is known to be a generalist forager and to capitalize on diverse foods, ranging from fruits and seeds to insects, small vertebrates, and carrion. In general, however, Culpeos favor small mammals (Lozano *et al.* 2024), and within this group they often forage selectively, favoring some species over others (*e. g.*, Jaksic *et al.* 1992, 1993, this study), and while other studies have demonstrated numerical responses to variation in key prey species, this is the first to document functional responses. At Las Chinchillas National Reserve, located in the Andean foothills less than 100 km from Fray Jorge, Jaksic *et al.* (1992) showed that the diet of Culpeos and Chillas continued to emphasize *A. bennettii* and *O. degus* through an irruption of *A. olivacea* and *P. darwin*i; foxes there consumed the latter two species in proportion to availability. In earlier work at Fray Jorge, Culpeos "showed strong prey preferences for some mam-

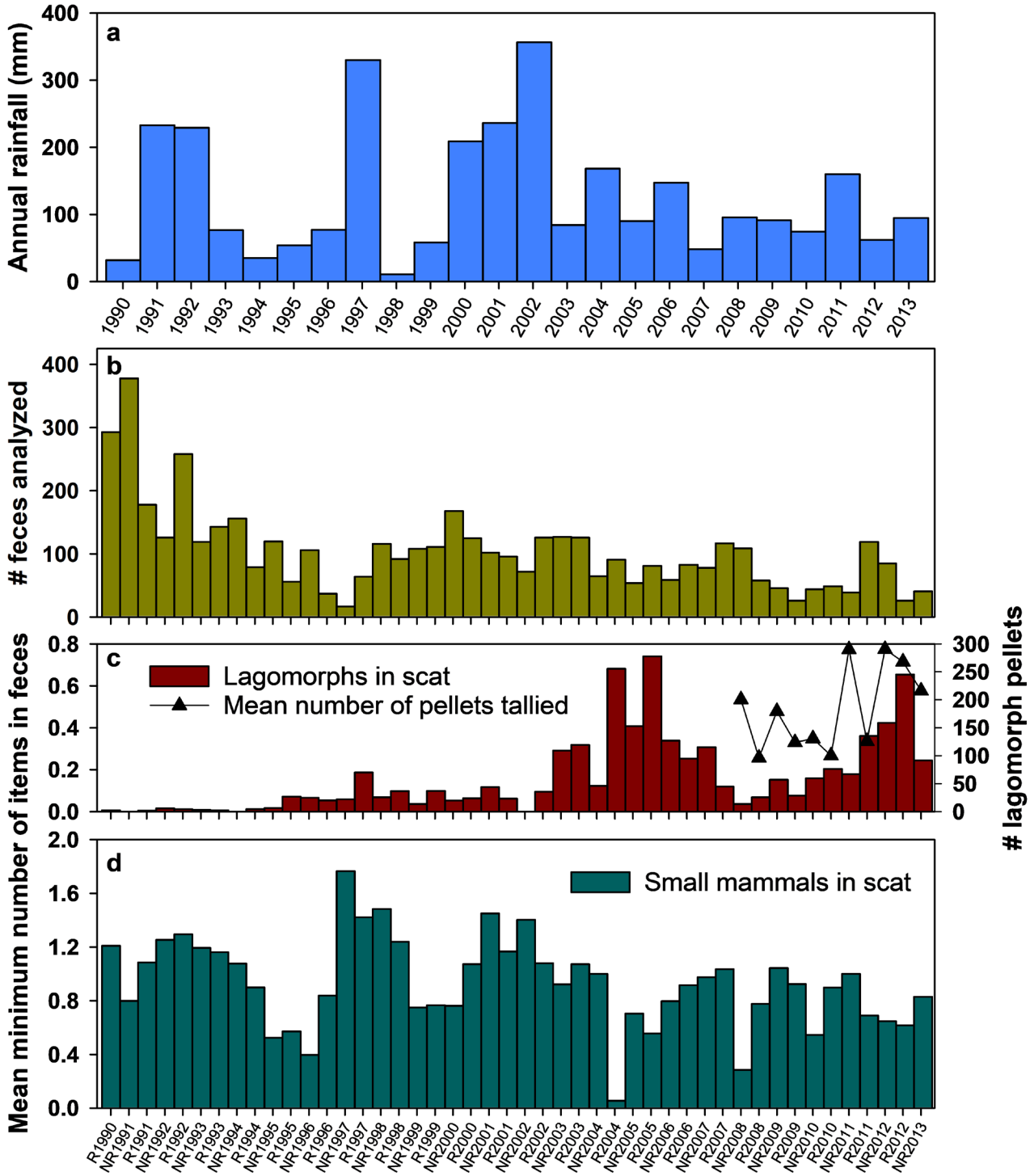

Figure 6. The importance of invasive lagomorphs (*Lepus europaeus* and *Oryctolagus cuniculus*) in the diet of Culpeo (*Lycalopex culpaeus*) became apparent in 2003 (a), following three sequential rainy years and a 2002 outbreak of canid distemper that killed an undetermined portion of the local fox population (Moreira and Stutzin 2005, Acosta Jamett 2010). The number of small mammals (b) fluctuated temporally but did not covary (positively or negatively) with that of lagomorphs. Triangular symbols in panel c present the mean number of lagomorph pellets tallied on standardized stations from 2008 through 2013 (see methods).

malian species [most notably, *Abrocoma*], regardless of their abundance in the field, and thus failed to display functional responses" (Jaksic *et al.* 1993:305). After an irruption of small mammals following ENSO-associated rains in 1991, Culpeos at Fray Jorge "appeared to select *O. degus*" (Jaksic *et al.* 1997:346). These studies were based on limited time series, however, and discrepancies with results presented here (using over two decades of data) support a caveat by Jaksic *et al.* (1996:252) "that short-term studies of species assemblages be used cautiously."

Numerical responses to variation in small mammal abundance are clear from our data (Figures 1 and, 4). What is novel here, however, is the demonstration of a Type II functional response as well (Table 1, Figure 5). Culpeos show a clear increase in per capita consumption as prey population sizes increase (Table 1, Figure 5). Further work will be needed to clarify how this response varies across prey species, but data from both Fray Jorge and Las Chinchillas suggest that Culpeos are likely to select *Abrocoma* and *O. degus*, although data presented here suggest that the latter may be abundant in the diet of Culpeos merely because they are abundant in the landscape. As noted above, these are two of the largest species of rodents in the region, and therefore should provide the greatest energetic value.

A generalist predator would be expected to exhibit functional responses to variation in other prey as well (*e. g.*, non-mammalian prey). Our data support such a conjecture, as the proportion of non-mammalian prey in some years (*e. g.*, 1990 to 91, 1994 to 96, 1999 to 2002, 2004, 2006 to 11) increases markedly (Figure 2b), and even seed consumption (Figure 2a) varies notably over time. These functional responses are more difficult to characterize, however, as we lack data on availability for these food items, and diagnostic characteristics often do not appear in scat. However, it appears that the generalist Culpeo, like many other small canids, readily diversifies its diet under in the face of variation in food availability.

The role of spatial heterogeneity. While our research on small mammal assemblages (and hence our estimates of prey availability) is restricted largely to thorn scrub habitat, Fray Jorge includes other distinct habitats as well, and these appear to serve as source habitats for some small mammal species (Milstead *et al.* 2007). Perhaps most notably, moist habitat occurs where the water table remains high enough to support mesic vegetation (*e. g.*, aguadas, which occur at the bottom of valleys), and coastal fog deposits sufficient moisture on higher elevations of coastal ranges (*e. g.*, the Altos de Talinay) to support patches of forest reminiscent of Valdivian rainforest of south-central Chile (Villagrán *et al.* 2004, Squeo *et al.* 2016). The former habitat appears to be key for species such as *O. longicaudatus* and likely *Abrocoma*, whereas the latter is a source for both *A. longipilis* and *O. longicaudatus*, both of which "spill over" to our study plots in thorn scrub during wet years when source populations experience growth (Milstead *et al.* 2007).

A second species of *Octodon* also occurs in moister habitats in Fray Jorge and merits further consideration; *O. lunatus* is the second largest small mammal at Fray Jorge, so we assume it would be favored when available. We tallied 101 individuals of *O. lunatus* in scat collected during 25 of 46 seasons analyzed here (in contrast to 1,148 individuals of *O. degus* in 45 of 46 sessions), but we captured this species in our monthly trapping efforts only in 11 seasons (and never in abundance), presumably reflecting habitat preferences. Ancillary trapping in aguada habitats supports a hypothesis that *O. lunatus* is more prevalent here than in thorn scrub (Milstead *et al.* 2007), but given the lack of data on availability we have excluded this species from analysis here. Further efforts to characterize demographic patterns in these key habitats, and to tie these to predator behavior and foraging, would be highly instructive.

Are foxes turning to lagomorphs? Although lagomorphs have been present in Culpeo scat through most of our study, it is only since about 2003 that they have contributed substantially to scat composition (Figures 2 and 6). The increase in lagomorph remains in Culpeo scat in 2003 to '07 followed both a series of high rainfall years (2000 to '02) and a 2002 to '03 outbreak of distemper in Culpeos at Fray Jorge, so it is difficult to determine which of these influences led to the apparent increase in consumption of lagomorphs, although lagomorphs increased in prevalence at Las Chinchillas at about the same time (F. Jaksic pers. comm. to DAK, October 2016), suggesting that the elevated rainfall influenced lagomorph abundance regionally. We initiated surveys of lagomorph activity in 2008 (symbols in Figure 6a), and limited data since then are suggestive of a relationship between lagomorph abundance and their presence in fox scat, although additional data are needed to resolve this association. However, such a delayed response to the presence of invasive lagomorphs by Culpeos would reflect similar observations for Black-chested Buzzard Eagles (*Geranoaetus melanoleucus*—Pavez *et al.* 2010). We predict that Culpeos will increase their consumption of invasive lagomorphs, especially the more abundant *Oryctolagus*, during years of high rabbit abundance or low small mammal abundance.

More on the value of long-term data. In the first decade of this project, Degus were positively selected by Culpeos in 15 of 22 6-mo periods (Figure 3). It was during this period that Jaksic *et al.* (1993) reported that Culpeos exhibited strong preferences for certain prey, and these preferences did not vary with fluctuations in different prey species, thereby displaying no functional response. A slightly longer database allowed Jaksic *et al.* (1997) to document an apparent functional response, as Culpeos shifted from *Abrocoma* (which they generally favored) towards Degus as the latter increased in abundance.

These earlier analyses were undertaken well before an apparent change in climatic patterns (*ca.* 2001/02) that led to local dominance by Degus (Meserve *et al.* 2011, 2016). From about 2001 through the period reported here (a

mega-drought—CR2 2015, Garreaud *et al.* 2020), Degus comprised 60 % or more of the small mammal biomass at Fray Jorge; oddly, throughout this time (*e. g.*, since 2001NR in Figure 3g) Degus were negatively selected in all but four periods (*e. g.*, 21 of 25 6-mo periods), representing an almost symmetrical pattern relative to that observed prior to this period, when Degus were consumed in excess of apparent availability. This change may reflect the apparent increase in consumption of lagomorphs (compare *Octodon* selection in Figure 3g against the presence of lagomorphs in scats in Figures 2 and 6). Regardless, it is presumably the increased resolution provided by the unusually long-term window of observation here that has allowed us to detect such a clear functional response to changes in small mammal numbers. Further work is needed to dissect this functional response among component small mammal species.

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Wildlife disease surveillance from village to peak: Trypanosome infections of mammals on Sulawesi revealed higher prevalence in intact montane forests

Amy L. Adams[1], Anang S. Achmadi[2], Ahmad Mursyid[3], Heru Handika[4,5], M. Rizaldi T.J.P. Nurdin[6], Jacob A. Esselstyn[4,5], Susan L. Perkins[7], Karen M.C. Rowe[1,8], and Kevin C. Rowe[1,8]*

[1] Sciences Department, Museums Victoria Research Institute, Carlton 3053, Melbourne. Victoria, Australia. Email: aadams@museum.vic.gov.au (ALA), karowe@museum.vic.gov.au (KMCR), krowe@museum.vic.gov.au (KCR).

[2] Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, Baden Riset dan Innovasi Nasional. Cibinong, Indonesia. Email: gudelly@gmail.com (ASA).

[3] The Indonesian Society for Bioinformatics and Biodiversity (MABBI). Malang, Indonesia. Email: mursyid.au@gmail.com (AM).

[4] Museum of Natural Science, Louisiana State University, Baton Rouge. Louisiana 70803, USA. Email: hhandi1@lsu.edu (HH), esselstyn@lsu.edu (JAE).

[5] Department of Biological Sciences, Louisiana State University, Baton Rouge. Louisiana 70803, USA. Email: hhandi1@lsu.edu (HH), esselstyn@lsu.edu (JAE).

[6] Department of Biological Education, Universitas Sulawesi Barat, Majene. West Sulawesi, 91411, Indonesia. Email: rizalditrias@unsulbar.ac.id (MRTJPN).

[7] Department of Biology, The City College of New York, New York, NY 10031, USA. Email: sperkins@ccny.cuny.edu (SLP).

[8] School of Biosciences, The University of Melbourne, Parkville, Victoria, 3010, Australia. Email: krowe@museum.vic.gov.au (KCR), karowe@museum.vic.gov.au (KMCR).

* Corresponding author: https://orcid.org/0000-0002-1215-9153.

Zoonotic diseases, including those carried by mammalian hosts, pose a significant threat to human health worldwide and substantial investment in wildlife disease surveillance is aimed at identifying the risk of spillover from wildlife to human populations where they interact. However, host species diversity is highest in the most intact habitats away from human habitation and most of the potential host species within these habitats are unsampled for infections. This is particularly true in biodiverse tropical ecosystems where the prevalence and identity of infections are the least known. We screened for presence of trypanosomes in 2,335 specimens from 66 species of rodents and shrews sampled from 11 mountain areas on the tropical island of Sulawesi, Indonesia. Our sampling spanned from the edge of human occupation into the most intact forests available on the island with sampling elevations ranging from 220 to 2,700 m. The two most common *Trypanosoma* species we detected were a native species from the Theileri clade (19.0 % of samples) and an introduced species from the Lewisi clade (5.1 % of murid rodent samples). Both species were detected at all elevations, extending from village edges to mountain peaks, but both reached their highest prevalence above 2,000 m elevation in the most intact forest away from human habitation. If these patterns with trypanosome infections are typical of other zoonotic diseases, wildlife disease surveillance would need to shift resources to study host-pathogen dynamics in more remote ecosystems. Sampling focused on the breadth of biodiversity, such as collected by and housed in natural history collections, is needed to further our understanding of zoonotic diseases and their prevalence.

Las enfermedades zoonóticas, incluidas las transmitidas por mamíferos hospedadores, plantean una amenaza importante para la salud humana en todo el mundo y se ha realizado una inversión sustancial en la vigilancia de enfermedades de la fauna silvestre para identificar el riesgo de contagio de la fauna silvestre a las poblaciones humanas con las que interactúan. Sin embargo, la diversidad de especies hospedadoras es mayor en los hábitats más intactos alejados de la habitación humana y la mayoría de las especies hospedadoras potenciales dentro de estos hábitats no han sido muestreadas para detectar infecciones. Esto es particularmente cierto en los ecosistemas tropicales biodiversos donde la prevalencia e identidad de las infecciones son las menos conocidas. Analizamos la presencia de tripanosomas en 2.335 especímenes de 66 especies de roedores y musarañas muestreadas en 11 áreas montañosas de la isla tropical de Sulawesi, Indonesia. Nuestro muestreo abarcó desde el límite de la ocupación humana hasta los bosques más intactos disponibles en la isla, con elevaciones de muestreo que oscilaron entre 220 y 2.700 m. Resultados: Las dos especies de *Trypanosoma* más comunes que detectamos fueron una especie nativa del clado Theileri (19.0 % de las muestras) y una especie introducida del clado Lewisi (5,1 % de las muestras de roedores múridos). Ambas especies se detectaron en todas las elevaciones, desde los límites de las aldeas hasta los picos de las montañas, pero ambas alcanzaron su prevalencia más alta por encima de los 2000 m de altitud en el bosque más intacto alejado de la habitación humana. Si estos patrones con infecciones por tripanosoma son típicos de otras enfermedades zoonóticas, la vigilancia de enfermedades de la vida silvestre necesitaría reorientar los recursos para estudiar la dinámica huésped-patógeno en ecosistemas más remotos. Se necesita un muestreo centrado en la amplitud de la biodiversidad, como la recolectada y almacenada en colecciones de historia natural, para mejorar nuestra comprensión de las enfermedades zoonóticas y su prevalencia.

Keywords: Muridae; kinetoplastid; 18S ribosomal DNA (rDNA); parasite; prevalence; rodent; *Trypanosoma*; shrews; One Health.

Introduction

Zoonotic diseases are responsible for three-quarters of emerging infectious disease (EIDs) in humans, posing a significant threat to human health (Taylor *et al.* 2001; Jones *et al.* 2008). Over the last century, there has been an increase in the incidence of EIDs spilling over from animals (Taylor *et al.* 2001; Jones *et al.* 2008), with most of these originating from wildlife (Jones *et al.* 2008). The rise of zoonotic EIDs has been linked to changes in demographic and anthropogenic variables including human population growth and density as well as climate change (Patz *et al.* 2000, 2004; Wolfe *et al.* 2007; Jones *et al.* 2008). Changes to land use to support growing human populations can modify animal-human interactions and change transmission pathways of pathogens (Patz *et al.* 2000, 2004; Wolfe *et al.* 2007; Jones *et al.* 2008). EID events are predicted to continue to pose a significant threat to human health due to the ongoing growth of the human population and subsequent disturbance of natural ecosystems through further expansion of anthropogenic activities into previously unoccupied, intact, biodiverse habitats. The modification of biodiverse ecosystems alters the distributions and behaviour of parasites, their hosts and their vectors, which can increase disease spillover from animal to human populations (Patz *et al.* 2000, 2004; Wolfe *et al.* 2007; Jones *et al.* 2008, 2013; Karesh *et al.* 2012; Hassell *et al.* 2017; Wilkinson *et al.* 2018; Johnson *et al.* 2020).

Current wildlife disease surveillance studies are largely focused on urban to rural habitats, where human and wildlife interactions are greatest (*e. g.* Bell *et al.* 2018; Bird and Mazet 2018; Munyua *et al.* 2019; Nelson *et al.* 2019; Lushasi *et al.* 2020; Blasdell *et al.* 2022). However, most species diversity and associated zoonotic diseases are confined to intact and remote habitats away from major human activities (Keesing *et al.* 2010; Lawrence *et al.* 2018; Glidden *et al.* 2021). While bat diversity in tropical systems is highest in low elevations, even with human disturbance (Patterson *et al.* 1996; McCain 2007; Bogoni *et al.* 2021), other mammalian host species, such as small terrestrial mammals, reach peak diversity at mid- to high elevations, which are often farthest from human occupation and impacts (Brown 2001; Heaney *et al.* 2001; Rickart *et al.* 2001, 2011; McCain 2005). Increasing human encroachment on the dwindling remnants of intact, biodiverse ecosystems, particularly in the tropics, are likely to reveal numerous novel infections, some of which are pathogenic and cause diseases of significance to human and animal health (Jones *et al.* 2008; Keesing *et al.* 2010; Chan *et al.* 2013; Morand *et al.* 2019). The spread of pathogens both regionally and globally due to human activities is also leading to unique pathogen communities with novel disease interactions (Kreuder Johnson *et al.* 2015). Due to limitations in detecting pathogens within wild species, we have almost no understanding of those dynamics (Johnson *et al.* 2020) including in intact ecosystems where most host diversity lies. Further research is required to assess the risk of EIDs from within these ecosystems.

The genus *Trypanosoma* (Euglenozoa: Kinetoplastea) comprises a diverse range of protozoan blood parasites, including zoonotic species, with a wide host range in all vertebrate classes. Despite their ubiquity, there is substantial variation in their geographic range sizes (locally endemic to cosmopolitan), host specificity (specific to generalist), morbidity (benign to lethal), and association with humans (intact to disturbed habitats; Simpson *et al.* 2006; Hamilton *et al.* 2007; Smith *et al.* 2008; Thompson *et al.* 2014a; Cooper *et al.* 2017; Yazaki *et al.* 2017). For example, some trypanosome species have a long natural association with their host species with limited evidence of morbidity (Hoare 1972; Smith *et al.* 2005; Averis *et al.* 2009). Other species have been spread globally by recent human-mediated dispersal activities, resulting in disease impacts on wildlife or humans (Joshi *et al.* 2005; Hamilton *et al.* 2007; Verma *et al.* 2011; Truc *et al.* 2013; Thompson *et al.* 2014b; Pumhom *et al.* 2015). Novel host interactions and co-infection with non-native species can lead to increased host morbidity by trypanosomes which are normally considered benign and which can cause atypical infections in humans (Smith *et al.* 2008; Wyatt *et al.* 2008; Botero *et al.* 2013; Desquesnes *et al.* 2013; MacPhee and Greenwood 2013; Milocco *et al.* 2013; Truc *et al.* 2013; Pumhom *et al.* 2014; Cooper *et al.* 2017). These novel interactions of native and introduced trypanosomes provide an opportunity for investigating how human encroachment, both through introducing parasites and encountering native parasites, influences the prevalence of zoonotic infections in intact ecosystems (Wood *et al.* 2014; Salzer *et al.* 2016; Faust *et al.* 2018; Morand *et al.* 2019).

Trypanosomes are readily detected by molecular DNA screening of tissues or blood from host animals (Clausen *et al.* 1998; Noyes *et al.* 1999; Desquesnes and Dávila 2002; Cummings and Tarleton 2003). While most species are defined by morphology and other phenotypes inferred through microscopy, trypanosomes that infect mammals are broadly classifiable into 4 clades inferred from 18S rRNA (Hamilton *et al.* 2007). The clades, which include numerous species yet to be formally described, are named after the most widespread and significant trypanosome species (Hoare 1972; Simpson *et al.* 2006; Hamilton *et al.* 2007; Milocco *et al.* 2013; Thompson *et al.* 2014b; Pumhom *et al.* 2015; Cooper *et al.* 2017). The Theileri clade contains trypanosome species infecting a wide range of mammalian hosts and is named for the cosmopolitan bovid-infecting *T. theileri* (Hoare 1972; Hamilton *et al.* 2005a; Rodríguez *et al.* 2010). While trypanosomes in the Theileri clade are typically considered non-pathogenic, pathogenic cases have been reported in animals (*e.g.* Amato *et al.* 2019). The Lewisi clade contains trypanosome species that typically infect rodents, and is named for *T. lewisi*, which has been spread globally with the introduction of black rats, *Rattus rattus* (Hoare 1972; Hamilton *et al.* 2005b, 2007). While normally infecting rodents and non-pathogenic, *T. lewisi* infections of humans have been reported with clinical implications (*e. g.* Truc *et al.* 2013). The Cruzi clade contains trypanosomes pri-

marily from bats, but with several trypanosomes switching to other terrestrial mammalian hosts particularly in South America and Australia (Hamilton *et al.* 2012). These widespread trypanosomes are largely host-specific with some pathogenic species such as *T. cruzi*, which causes Chagas disease in humans (Hamilton *et al.* 2007, 2012; Botero *et al.* 2016; Cooper *et al.* 2017). The Brucei clade contains pathogenic trypanosomes, including *T. brucei*, which causes African sleeping sickness. Originating from African mammals, these trypanosomes now infect a broad range of mammalian host species on most continents (Hamilton *et al.* 2005, 2007; Rodrigues *et al.* 2006; Desquesnes *et al.* 2013).

The oceanic island of Sulawesi, Indonesia is a biogeographic crossroads between Asia and Australia that has not been connected to either for at least 50 million years. The single island of Sulawesi formed from several smaller islands over the last 2 to 5 million years (Whitten *et al.* 1987; Hall 2013; Nugraha and Hall 2018; Rowe *et al.* 2019). It is the largest landmass in the vast archipelago that spans > 1,000 kms between the Asian and Australian continental shelves. Most of Sulawesi's fauna are endemic (*i. e.* native only to the island), with distributions often restricted to specific areas on the island (*e. g. Bunomys coelestis*). These faunal communities are composed of a globally unique assemblage of Asian- and Australian-derived lineages (Michaux 2010; Lohman *et al.* 2011; Stelbrink *et al.* 2012). Sulawesi is also home to more than 20 million people (> 100 per km^2) and most of its intact pristine forests are found at higher elevations on its many rugged mountains that reach as high as 3,478 m. The vast majority of Sulawesi's human population and domestic livestock live at elevations below 1,500 m, resulting in < 20 % of lowland forests being in good condition (Supriatna *et al.* 2020). In contrast, 70 % of forests above 1,500 m are considered in good condition (Cannon *et al.* 2007).

Sulawesi is also home to both native and introduced species of trypanosomes including species from the Theileri, Lewisi and Brucei clades (Luckins 1998; Winterhoff *et al.* 2020; Setiawan *et al.* 2021). Trypanosome species from the Theileri clade that are closely related to *T. cyclops* have been detected infecting a wide range of mammalian hosts on the island, including rodents, bats, and shrews, often with high prevalence and are therefore presumed native (Weinman 1972; Winterhoff *et al.* 2020; Mursyid *et al.* 2023). Lewisi-clade trypanosomes, introduced with rodents brought by humans, have been detected infecting both native and introduced rodent species on Sulawesi (Winterhoff *et al.* 2020). *Trypanosoma evansi*, of the Brucei clade and responsible for the major disease known as surra in animals, originated from Africa and has been detected infecting livestock such as cattle, buffaloes and horses on Sulawesi (Luckins 1998; Desquesnes *et al.* 2001, 2013; Setiawan *et al.* 2021). Sulawesi's position between the Asian and Australian continents, suggests that it is an important link in the biogeography of trypanosomes including within the Theileri clade (Stelbrink *et al.* 2012; Winterhoff *et al.* 2020). Nearly identical sequences to the named species *T. cyclops*, described from an infection in a Malaysian primate (Weinman 1972), have been detected from Asia to Australia in rodents and marsupials (Thompson *et al.* 2014b; Cooper *et al.* 2017; Winterhoff *et al.* 2020). These may represent a widespread species or one or more undescribed species. Sulawesi therefore offers an opportunity to undertake wildlife disease surveillance from disturbed through to intact habitats using trypanosomes as disease indicators.

In this study, we screened blood samples from 2,335 mammalian specimens collected over 10 years from 11 mountainous areas of Sulawesi spanning 220 to 2,800 masl. Our sampling starts from the last human settlement and extends into the most intact habitats on each mountain, most of which occur at higher elevations. Here, we present trypanosomes as a model to examine how infections by introduced and native parasites are distributed among disturbed and intact habitats along elevational gradients on a human-dominated tropical island. We predicted that invasive Lewisi clade trypanosomes would occur more frequently at lower elevations coinciding with more disturbed habitats, whereas native trypanosomes of the Theileri clade (*T. cyclops*) would occur more frequently at higher elevations coinciding with less disturbed habitats, mirroring the patterns among native and introduced mammals. We also predicted that the co-occurrence of introduced and native trypanosomes would be most common at the interface of disturbed and intact habitats. The samples used in this study are accompanied by numerous additional specimens lodged in Indonesian, American and Australian natural history collections that highlight the vast value of natural history collections for surveillance of wildlife disease in the most remote and biodiverse regions of our planet (Dunnum *et al.* 2017; Colella et al. 2021; Thompson *et al.* 2021).

Materials and methods

Sample collection. From 2010 to 2019, we surveyed rodents (families Muridae and Sciuridae) and shrews (family Soricidae) at 11 localities across Sulawesi, Indonesia (Figure 1; Table 1; Supplementary Material S1). The localities sampled spanned 5.3 ºS to 1.06 ºN extending from the southern end of the southwest peninsula to the eastern end of the northern peninsula. At each locality, we surveyed as wide a range of elevations as possible with a mean elevation range spanning 1,267 m per locality (Table 1). The lowest elevation sampled at each locality, typically near the edge of the last human settlement, ranged from 220 to 1,660 m (mean 632 m) and the highest elevation sampled ranged from 450 to 2,804 m (mean 1,899 m). Intact habitats comprised primary forest with mixed species and mixed age stands without evidence of ongoing or past extractive forest practices. Disturbed habitats comprised secondary or degraded forest resulting from human modifications including agriculture, plantations, timber clearing, and/or mining activities. Lowland habitats (< 1,000 m) were once dominated by lowland rainforest that when surveyed was in different degrees of conversion to plantations or villages, and/or recovering

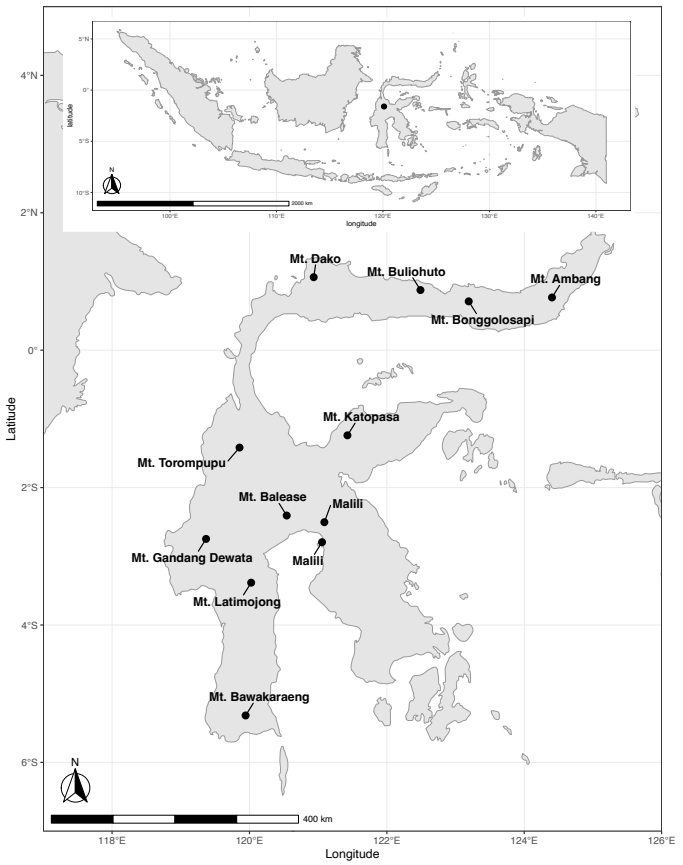

Figure 1. Sampling took place on the Indonesian island of Sulawesi (inset). The 11 surveyed localities are labelled by the nearest mountain. Surveys near the town of Malili sampled 2 nearby lowland locations, the results of which were combined for analyses.

from disturbance as secondary growth. Only four localities contained intact forest below 1,000 m and only one locality, Mt. Bonggolosapi within Bogani Nani Wartabone National Park, contained intact forest below 500 m. Highland habitats (> 1,000 m) were dominated by intact forest. Disturbed habitat above 1,000 m was only sampled at two localities, including the highest elevations on Mt. Bawakaraeng where a popular camping area was heavily disturbed.

Surveys at each locality lasted an average of 21 days (4 to 38 days) and employed a mix of live traps, snap traps, and 20 to 30 L pitfall buckets. Surveys were led by Museum Zoologicum Bogoriense (MZB), Bogor, Indonesia where most specimens were deposited. Remaining specimens were deposited in Museums Victoria (NMV), Melbourne, Australia; Museum of Vertebrate Zoology (MVZ), Berkeley, USA; Field Museum of Natural History (FMNH), Chicago, USA; and Louisiana State University Museum of Natural Science (LSUMZ), Baton Rouge, USA. Survey methods were approved by institutional animal care and use committees at US institutions, the animal ethics committee at Museums Victoria and complied with all Indonesian laws and regulations. Specimens were prepared as vouchers including skins, skeletal material, and fluid-preserved (formalin or ethanol) whole animals. In addition, a series of biological samples were preserved in ethanol, RNA later, and/or liquid nitrogen for each specimen, including liver, kidney, spleen, lung, heart, muscle, blood, adrenal gland, preputial gland, gastrointestinal contents, and faeces. Blood films were collected from specimens starting from 2016.

DNA extraction and sequencing. Total genomic DNA was extracted mainly from liver tissue (n = 2,085) but also from spleen (n = 79), muscle (n = 6), kidney (n = 1), or from an unknown tissue type (n = 164; Supplementary Material S1). Extractions were performed using a salting method, a QIAGEN DNeasy blood and tissue kit (QIAGEN Inc, Valencia, CA, USA), a Wizard SV 96 Genomic DNA Purification System kit (Promega Corporation, Madison, WI, USA), or a QIAextractor (DX reagents and plasticware) following the manufacturer's protocols.

DNA samples were screened for Kinetoplastea infections at the 18S subunit of the rRNA locus using a set of nested trypanosome-generic primers: SLF/S762R and S823F/S662R following the previously described PCR protocol (Winterhoff *et al.* 2020; Supplementary Material S2). Conditions for both primary and nested PCR reactions included: a 95°C initial pre-amplification step for 5 min, fol-

Table 1. Sampling information and PCR screening results of Kinetoplastea infections in rodents and shrews for each locality surveyed on Sulawesi.

Locality	Year(s) sampled	Latitude (Centroid)	Longitude (Centroid)	Elevation range of surveys (masl)	Survey Days	Samples screened	Screened host genera (species)	Theileri Clade (*T.cyclops*)	Lewisi Clade	Cruzi Clade	Other Kinet.
Malili	2010	(a) -2.79438 (b) -2.50368	(a) 121.05643 (b) 121.09012	450	4	47	4 (6)	6	0	0	0
Balease	2010	-2.40689	120.54211	900 – 1150	15	71	6 (11)	5	1	0	1
Gandang Dewata	2011; 2012	-2.74695	119.36802	220 - 2670	44	212	17 (26)	46	21	4	1
Latimojong	2011; 2016	-3.38414	120.02442	683 - 2535	27	319	11 (21)	141	26	0	1
Dako	2013; 2018	1.06213	120.93513	310 - 2230	38	516	10 (23)	111	12	0	1
Buliohuto	2014	0.87505	122.48904	400 - 1390	16	72	6 (9)	0	6	0	5
Bawakaraeng	2016	-5.31685	119.94390	1660 - 2804	15	349	9 (17)	38	11	0	7
Ambang	2016	0.76675	124.40372	1472 - 1655	21	90	7 (12)	6	0	0	1
Katopasa	2017	-1.23955	121.42607	220 - 2575	20	232	5 (20)	75	5	2	1
Torompupu	2017	-1.41764	119.85558	410 - 2210	19	173	8 (21)	13	3	0	1
Bonggolosapi	2019	0.71088	123.19154	228 - 1216	15	254	9 (18)	1	5	6	2
Total						**2,335**	**23 (65)**	**442**	**90**	**12**	**21**

lowed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 2 min and 20 s; and a final extension step at 72°C for 7 min. A known positive control was used in each PCR reaction to determine successful amplification. All reactions were performed in an Eppendorf Mastercycler Nexus GSX1. PCR amplicons were visualised on a 1.2 % agarose gel, stained with SYBR safe (Invitrogen, USA). Each PCR-positive sample was purified using ExoSAP (USB Corporation, Cleaveland, Ohio, USA) and both strands were sequenced via automated DNA Sanger sequencing on an ABI 3730xl DNA Analyzer at Macrogen Inc (Seoul, Korea). Sequences were edited and aligned in CodonCode v.5.1.5 (CodonCode Corporation, Dedham, MA, USA) and manually inspected in Aliview v.1.23 (Larsson 2014). All sequences were identified to genus and species where possible using the nucleotide BLAST tool within the NCBI GenBank database. DNA sequences are available in GenBank (Supplementary Material S1). Due to the sensitivity of PCR methods, detection of a Kinetoplastea infection does not necessarily indicate an active replicating infection. Rather prevalence reflects the percentage of individuals that have signs of being or have previously been infected, including transient infections.

Microscope analysis. Microscopic observation of parasite morphology is a valuable tool assisting the taxonomic resolution and identification of trypanosome taxa. We collected and prepared blood smears in the field from already deceased animals which can decrease the quality of the prepared smear (*i. e.* thick, coagulated smears) from mid-2016 onwards. Slides were stored in a box with silica desiccant for up to four weeks without fixation. Upon return to the laboratory, smears were fixed with 100 % methanol and stained with a 10 % Giemsa solution.

We compared the efficiency between PCR protocols and microscopy at detecting trypanosome infections, as well as confirming circulation of trypanosome infections in the blood by screening a random sub-set of blood smears (n = 120 PCR-positive, 40 PCR-negative) collected between 2017 and 2018 using light microscopy at x400 and x1,000 magnification for 15 minutes. All blood smears are registered in the Museums Victoria collection and linked to specimens registered at Museum Zoologicum Bogoriense.

Trypanosome prevalence. We tested the null hypotheses that prevalence for each trypanosome clade did not differ between habitat types (intact or disturbed) or among elevation bins. Because forest types and elevation bins are not independent, with disturbed forest dominating lower elevation and intact forest dominating higher elevation, we are not able to disentangle their respective effects on prevalence. We present both to reveal if prevalence appears higher in disturbed/low elevation habitats closer to human impacts as predicted or in intact/high elevation habitats farther from human interactions. We defined 'intact' forest as those exhibiting mixed species and mixed age stands without ongoing extractive forest practices and 'disturbed' as those showing signs of extractive forestry, including timber harvest, clearing and other signs of human impacts. Samples were categorised into 6 elevation bins: I) < 500 masl, II) 501 to 1,000 masl, III) 1,001 to 1,500 masl; IV) 1,501 to 2,000 masl, V) 2,001 to 2,500 masl, and VI) > 2,500 masl. These bins best represent the transitions between village and forest (I and II) and transitions among habitat with increasing elevation.

Testing each clade, we used Chi-square tests to compare prevalence. All Chi-square tests were conducted using the 'chisq.test' function in the *base* package of the R programming language (v. 4.3.1, R Core Team 2023). If our null hypotheses for forest types or elevation bins were rejected, we investigated which categories were significantly different by performing post-hoc analyses on the residuals using the 'chisq.posthoc.test' function in the same named package (v. 0.1.2, Ebbert 2019). We used the Bonferroni correction for multiple comparisons to control for the family-wise error rate.

Results

Sample collection. We collected a total of 3,307 rodent and shrew specimens from Sulawesi, of which we screened 2,335 for the presence of Kinetoplastea parasite infections. Our sampling of rats (family Muridae) included 44 endemic and two introduced species comprising 18 genera. Our sampling included all but seven endemic species of murids known from the island. Our sampling of squirrels (family Sciuridae) included all three genera endemic to the island, but included only three of the ten species (Musser *et al.* 2010). Our sampling of shrews (family Soricidae) included 17 of the 21 species in the genus *Crocidura* that are native to Sulawesi (Esselstyn *et al.* 2021) and one introduced species, *Suncus murinus*. We sampled and screened a mean of 16.8 rodent and shrew species (6 to 26 species) per locality (Table 2). From disturbed habitats we collected and screened a mean of 7.1 species per locality (2 to 16 species) whereas we collected and screened a mean of 16.6 species from intact habitats (9 to 23 species). Per elevation bin we collected and screened a mean of 8.6 species (7.3 to 11 species) with the highest number of rodents and shrew species collected from bins IV (1,501 to 2,000 masl) and V (2,001-2,500 masl). Screened specimens included 18 genera from Muridae (1,779 specimens), three genera from Sciuridae (38 specimens) and two genera from Soricidae (519 specimens; Table 3).

DNA extraction and sequencing. We detected DNA from 3 Kinetoplastea orders in 24.2 % of our samples (n = 565, Table 4). Trypanosomes (*Trypanosoma*: Trypanosomatidae: Trypanosomatida) comprised > 96 % of detections and were detected in 23.3 % of our samples including 65.2 % of the genera and 69.8 % of the species sampled. These comprised 29 species of murid rodents, 15 shrew species, and 2 squirrel species (n = 544; 439 rats, 103 shrews, 2 squirrels; Tables 1, 3, 4). Trypanosomes were detected in 513 liver (24.6 %), 4 spleen (5.1 %), and 27 unknown tissue (16.5 %) samples. In all but 2 species without PCR-positive detections (n = 20), the sample sizes were small (n = 1-11; $\bar{x}$ = 4.5).

Table 2. Observed richness of host species (rodents and shrews) across localities, habitat types and elevation bins. Elevation bins for each locality are shaded to indicate disturbed habitat (dark grey), intact habitat (light grey), mixed habitat (light grey with black box). For mixed habitat bin, richness is separated for disturbed (left of forward slash) and intact (right of forward slash) based on individual traplines. Available but unsampled elevation bins at each locality are shaded without numbers.

Locality	Richness (Total)	Richness Disturbed	Richness Intact	Richness per elevation bin					
				I	II	III	IV	V	VI
				<500 m	500-1,000 m	1,001-1,500 m	1,501-2,000 m	2,001-2,500 m	>2,500 m
Malili	6	6	NA	6					
Ambang	12	NA	12			12	8		
Balease	11	NA	11		11	5			
Bawakaraeng	17	5	17				15	12	5
Bonggolosapi	18	6	17	6/14	3	6			
Buliohuto	9	4	9	4	7	6			
Dako	23	16	21	9	14	15	18	8	
Gandang Dewata	26	7	23	6	1		18		15
Katopasa	21	11	15	10	5	12	7	6	3
Latimojong	21	7	20		4	3	1	22	6
Torompupu	21	2	21	2	14	5	10	6	
Mean	16.8	7.1	16.6	7.7	7.4	8	11	10.8	7.25

Based on the 18S rDNA gene sequences, we identified infections by trypanosomes from three of the four clades that infect mammals (Cooper *et al.* 2017); the Theileri, Lewisi and Cruzi clades (Tables 3, 4). All sequences within the Theileri clade matched closely (> 97 % sequence similarity) to *T. cyclops*, described from Malaysian macaques, and which have previously been detected in rodents, marsupials, frogs and terrestrial leeches from Australia, Sulawesi and Asia (Cooper *et al.* 2017; Winterhoff *et al.* 2020). Sequences within the Lewisi clade were nearly all identical and had a > 98 % sequence similarity with several named species of *Trypanosoma* but which cannot be distinguished solely by 18S rDNA sequences. Species included *T. lewisi, T. otospermophili*, *T. microti*, *T. musculi*, and *T. rabinowitschae* (see phylogeny in Winterhoff *et al.* 2020). One recovered sequence shared 100% similarity to *T. lewisi* previously detected in rodents from the Philippines and Sulawesi (Mafie *et al.* 2019). One sequence within the Cruzi clade matched closely with the rodent-infecting *T. conorhini* (99 % sequence similarity). The remaining sequences within the Cruzi clade had a 97 to 98 % sequence similarity with *T. terrestris* which has been detected infecting tapirs (Table 4).

Several other either free-living or exclusively arthropod-infecting kinetoplastid genera were also detected by PCR in our rodent and shrew samples but were not detected with blood smears (free-living genera: *Parabodo*, *Neobodo*, *Dimastigella*; monoxenous arthropod-infecting genera: *Blechomonas*, *Blastocrithidia*, *Herpetomonas*, *Kentomonas*; Table 4). The low detection rates, including several single infections, of these free-living or non-mammalian infecting genera probably does not reflect an infection in the mammalian specimens screened. The extremely low incidence of these genera suggests that our PCR protocols were mostly detecting the targeted mammalian-infecting Kinetoplastea species.

Microscopy screening. Trypanosomes were detected in 27 (16.9 %) of the blood smear slides from PCR-positive specimens (1 Cruzi clade, 3 Lewisi clade and 23 Theileri clade (*T. cyclops*) trypanosomes; Figure 2; see Supplementary Material S3). This may indicate false negatives in microscopy due to the lower sensitivity of this method at detecting active infections, especially at low parasitaemia levels (Setiawan *et al.* 2021), or false positives in PCRs where evidence of past infections can be detected due to the higher sensitivity of this method and therefore trypanosomes would not be observed in a blood film. False positives derived from past infections are irrelevant to our inference as our aim was to determine if an animal has been exposed to infection. Our low detection rates in microscopy may also be related to the difficulties in producing high-quality blood smears under challenging field conditions. Despite the often-poor quality of blood smears and low detection rates compared to PCR, trypanosome detections with microscopy confirm active infections in hosts and provide morphological references of PCR detections. Trypanosomes were not detected in the blood smears of PCR-negative blood specimens with false negatives less likely to occur from PCR than in microscopy (Valkiunas *et al.* 2008).

Trypanosome prevalence. Of the three clades of trypanosomes detected in this study the Theileri Clade was detected in 19.0 % of samples, Lewisi clade in 5.1% of samples and Cruzi clade in 0.5 % of samples. Theileri clade (*T. cyclops*) detections had a significantly higher prevalence than either Lewisi clade infections ($\chi^2 = 171.3$, *d.f.* = 1, $p < 0.001$) or Cruzi clade infections ($\chi^2 = 449.0$, *d.f.* = 1, $p < 0.001$). In relation to forest types, Theileri clade (*T. cyclops*) infections were significantly higher in intact forests (20.8 %) than in disturbed forest (7.3 %; $\chi^2 = 31.9$, *d.f.* = 1, $p < 0.001$; Figure 3a), whereas infection prevalences were similar between forest types for Lewisi clade infections of murids (intact = 5.2 %, disturbed = 4.2 %; $\chi^2 = 0.34$, *d.f.* = 1, $p = 0.562$; Figure 3b; see Appendix I). Cruzi clade (Terrestris-like) infections were significantly higher in disturbed forest (1.8 %) than in intact forests (0.3 %; CI: 1.71-24.2, *d.f.* = 1, $p = 0.003$).

Table 3. Sample sizes of PCR-screened rodent and shrew host species of Sulawesi. Number and percents of samples with PCR positives and detections of Theileri clade (*T. cyclops*) or Lewisi clade infections.

Order	Family	Genus	Species	Sample size		Theileri clade (*T. cyclops*)	Lewisi clade
				Screened	PCR positive *n* (%)	*n* (%)	*n* (%)
Rodentia	Muridae	*Bunomys*	*andrewsi*	131	54 (41.2%)	41 (31.3%)	11 (8.4%)
Rodentia	Muridae	*Bunomys*	*chrysocomus*	116	21 (18.1%)	14 (12.1%)	6 (5.2%)
Rodentia	Muridae	*Bunomys*	*coelestis*	64	21 (32.8%)	17 (26.6%)	4 (6.3%)
Rodentia	Muridae	*Bunomys*	*penitus*	121	84 (69.4%)	63 (52.1%)	20 (16.5%)
Rodentia	Muridae	*Bunomys*	*prolatus*	10	10 (100%)	10 (100%)	0 (0%)
Rodentia	Muridae	*Bunomys*	sp. unidentified	16	3 (18.3%)	2 (12.5%)	1 (6.3%)
Rodentia	Muridae	*Bunomys*	*torajae*	68	57 (83.8%)	49 (72.1%)	8 (11.8%)
Rodentia	Muridae	*Crunomys*	*celebensis*	2	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Echiothrix*	*leucura*	2	1 (50.0%)	1 (50.0%)	0 (0%)
Rodentia	Muridae	*Eropeplus*	*canus*	18	4 (22.2%)	3 (16.7%)	1 (5.6%)
Rodentia	Muridae	*Frateromys*	*fratrorum*	112	27 (24.1%)	23 (20.5%)	1 (0.9%)
Rodentia	Muridae	Genus	sp. nov.	11	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Gracilimus*	*radix*	4	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Haeromys*	*minahassae*	11	5 (45.5%)	1 (9.1%)	4 (36.4%)
Rodentia	Muridae	*Hyorhinomys*	*stuempkei*	3	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Lenomys*	*meyeri*	6	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Margaretamys*	*beccarii*	1	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Margaretamys*	*elegans*	2	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Margaretamys*	*parvus*	5	1 (20%)	0 (0%)	1 (20%)
Rodentia	Muridae	*Margaretamys*	sp. nov. 1	1	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Margaretamys*	sp. nov. 2	1	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Crunomys*	*dollmani*	10	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Crunomys*	*hellwaldii*	95	14 (14.7%)	3 (3.2%)	0 (0%)
Rodentia	Muridae	*Crunomys*	*musschenbroekii*	317	50 (15.8%)	45 (14.2%)	2 (0.6%)
Rodentia	Muridae	*Crunomys*	*wattsi*	27	14 (51.9%)	14 (51.9%)	0 (0%)
Rodentia	Muridae	*Melasmothrix*	*naso*	3	1 (33.3%)	0 (0%)	1 (33.3%)
Rodentia	Muridae	*Paucidentomys*	*vermidax*	2	1 (50%)	1 (50%)	0 (0%)
Rodentia	Muridae	*Rattus*	*bontanus*	36	4 (11.1%)	3 (8.3%)	1 (2.8%)
Rodentia	Muridae	*Rattus*	*exulans*	31	2 (6.5%)	0 (0%)	2 (6.5%)
Rodentia	Muridae	*Rattus*	*facetus*	49	4 (8.2%)	1 (2.0%)	2 (4.1%)
Rodentia	Muridae	*Rattus*	*hoffmanni*	163	26 (16.0%)	5 (3.1%)	20 (12.3%)
Rodentia	Muridae	*Rattus*	*marmosurus*	9	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Rattus*	*mollicomulus*	51	7 (13.7%)	0 (0%)	4 (7.8%)
Rodentia	Muridae	*Rattus*	*tanezumi*	1	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Rattus*	*xanthurus*	7	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Sommeromys*	*macrorhinos*	6	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*callitrichus*	7	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*celebensis*	20	1 (5%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*dominator*	149	12 (8.1%)	11 (7.4%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*hamatus*	1	0 (0%)	0 (0%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*punicans*	1	1 (100%)	1 (100%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*sp. nov. 1*	29	11 (37.9%)	11 (37.9%)	0 (0%)
Rodentia	Muridae	*Taeromys*	sp. nov. 2	4	1 (25%)	1 (25%)	0 (0%)
Rodentia	Muridae	*Taeromys*	*taerae*	41	15 (36.6%)	15 (36.6%)	0 (0%)
Rodentia	Muridae	*Tateomys*	*macrocercus*	7	2 (28.6%)	1 (14.3%)	1 (14.3%)
Rodentia	Muridae	*Tateomys*	*rhinogradoides*	8	1 (12.5%)	1 (12.5%)	0 (0%)
Rodentia	Sciuridae	*Hyosciurus*	*heinrichi*	4	1 (25.0%)	1 (25.0%)	0 (0%)
Rodentia	Sciuridae	*Prosciurillus*	*murinus*	33	1 (3.0%)	1 (3.0%)	0 (0%)
Rodentia	Sciuridae	*Rubrisciurus*	*rubriventer*	1	0 (0%)	0 (0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*australis*	5	2 (40.0%)	1 (20.0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*balete*	4	1 (25.0%)	1 (25.0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*brevicauda*	10	5 (50.0%)	5 (50.0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*caudipilosa*	45	7 (15.6%)	7 (15.6%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*elongata*	33	9 (27.3%)	9 (27.3%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*lea*	13	8 (61.5%)	8 (61.5%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*levicula*	19	1 (5.3%)	1 (5.3%)	0 (0%)

Table 3. Continuation.

Eulipotyphla	Soricidae	*Crocidura*	*mediocris*	21	0 (0%)	0 (0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*microelongata*	16	6 (37.5%)	6 (37.5%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*nigripes*	44	8 (18.2%)	8 (18.2%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*normalis*	5	0 (0%)	0 (0%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*ordinaria*	8	1 (12.5%)	1 (12.5%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*pallida*	28	6 (21.4%)	6 (21.4%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*parva*	84	4 (4.8%)	2 (2.4%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*pseudorhoditis*	66	21 (31.8%)	21 (31.8%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*quasielongata*	55	2 (3.6%)	1 (1.8%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	*solita*	42	23 (54.8%)	23 (54.8%)	0 (0%)
Eulipotyphla	Soricidae	*Crocidura*	sp. unidentified	19	4 (21.1%)	3 (15.8%)	0 (0%)
Eulipotyphla	Soricidae	*Suncus*	*murinus*	1	0 (0%)	0 (0%)	0 (0%)

Theileri clade (*T. cyclops*) infections were detected in 442 individuals representing 24 endemic murid, two squirrel, and 15 shrew host species and were detected from 10 of 11 localities sampled (Table 3; Figure 3a). No Theileri clade (*T. cyclops*) trypanosomes were detected in introduced species. Prevalence varied among taxa (range 2 % to 100%) and varied significantly among localities ($\chi^2 = 187.33$, *d.f.* = 10, $p < 0.001$; Figure 3a; see Appendix I). Prevalence was highest on Mount Latimojong (44.2 %, residuals = 9.46, $p < 0.001$) and infections were also significantly higher than average on Mount Katopasa (32.3 %, residuals = 4.32, $p < 0.001$). However, infections were significantly lower on Mount Bawakaraeng (10.9 %, residuals = -3.53, $p = 0.009$), Mount Torompupu (7.5 %, residuals = -3.45, $p = 0.013$) and Mount Bonggolosapi (0.4 %, residuals = -7.11, $p < 0.001$). No Theileri clade (*T. cyclops*) infections were detected on

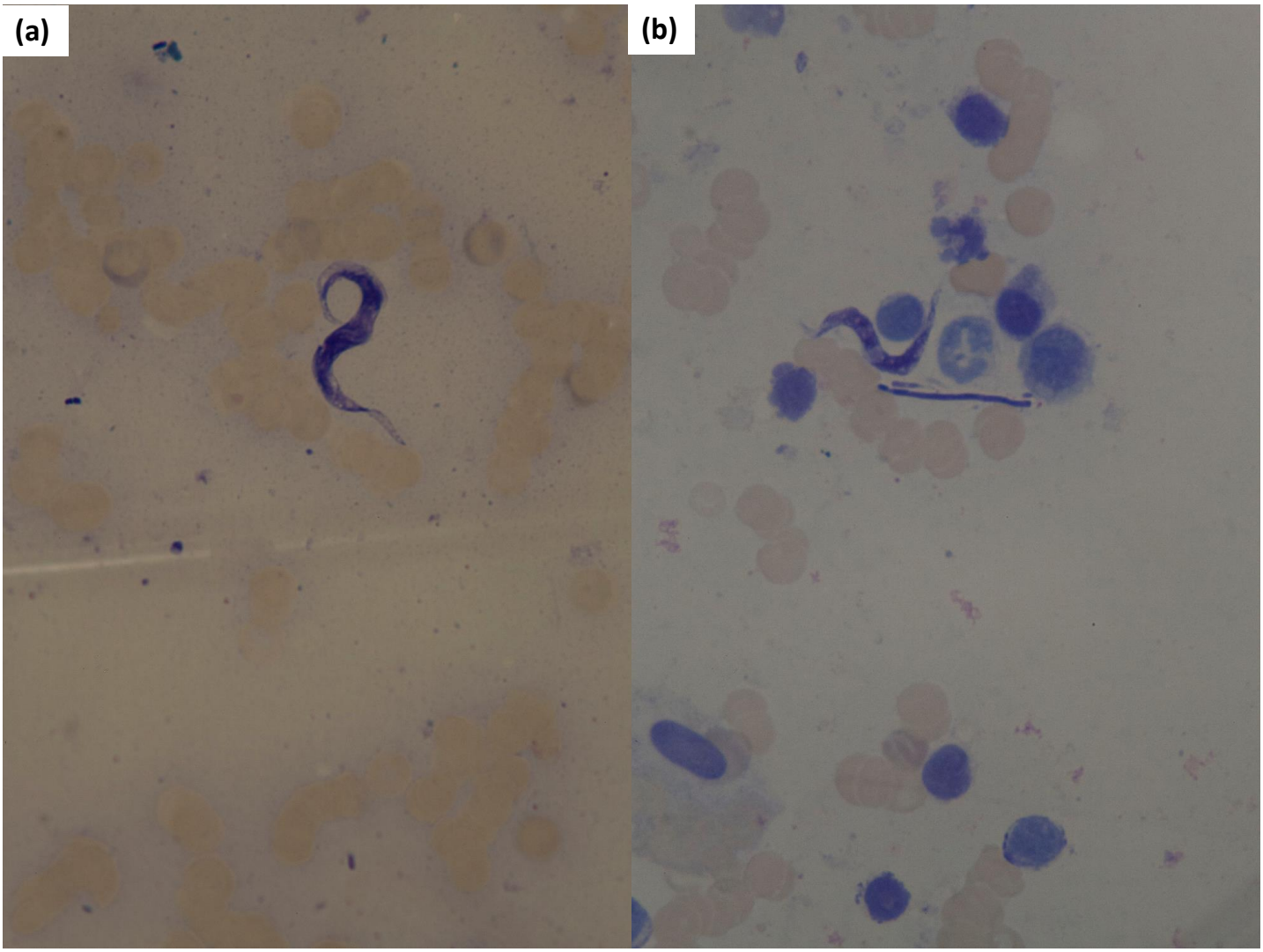

Figure 2. Blood from 2 *Bunomys chrysocomus* specimens illustrating examples of (A) Theileri clade (*T. cyclops*) and (B) Lewisi clade parasites among erythrocytes stained using 10% Giemsa stain under x1,000 magnification.

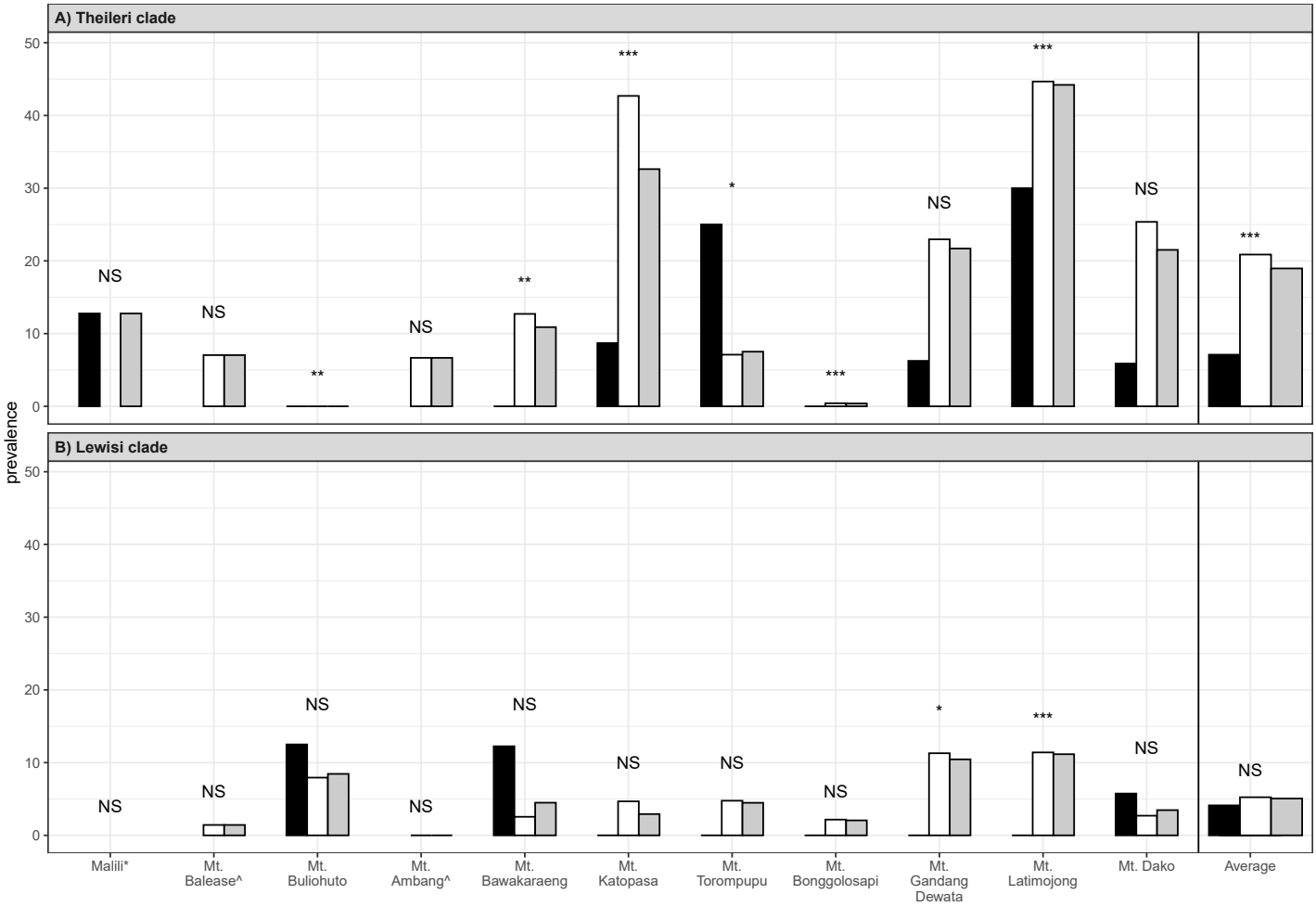

Figure 3. Prevalence of (A) Theileri clade (*T. cyclops*) and (B) Lewisi clade infections as detected by PCR in rodents and shrews by localities and habitat types on Sulawesi. Black and white bars represent prevalence in intact and disturbed habitats, respectively with overall prevalence in grey. Average prevalence by habitat type for all localities is included on the right. Localities where only disturbed (*) or intact (^) habitats were sampled are indicated. Symbols above the bars indicate significance (p value) of prevalence by locality and the average for that clade ('NS' = not significant, '*' = p = 0.01 – 0.05, '**' = p = 0.001 – 0.01, '***' = < 0.001).

Mount Buliohuto (0 %, residuals = 3.74, p = 0.004). There were no significant differences from the average prevalence of all sites for Malili (12.8%, residuals = -0.92, p = 1.00), Mount Ambang (6.7 %, residuals = -2.63, p = 0.19), Mount Balease (7.0 %, residuals = -2.26, p = 0.53), Mount Dako (21.5 %, residuals = 1.39, p = 1.00), and Mount Gandang Dewata (21.7 %, residuals = 0.88, p = 1.00).

For Theileri clade (*T. cyclops*) infections, prevalence varied significantly among elevation bins (χ^2 = 202.62, *d.f.* = 5, p < 0.001; Figure 4a; see Appendix I), with prevalence significantly higher than average in bin V (2,001 to 2,500 m; 37.0 %, residuals = 10.70, p < 0.001). Prevalence was significantly lower for the two lowest bins (bin I: < 500 m, 3.3 %, residuals = -8.65, p < 0.001; bin II: 501-1,000 m, 4.4 %, residuals = -6.87, p < 0.001). There were no significant differences from the average prevalence in bin III (1,001–1,500 m, 22.7 %, residuals = 1.87, p = 0.739) or bin IV (1,501–2,000 m, 21.1 %, residuals = 1.89, p = 0.713).

Lewisi clade infections were detected in 90 murid samples (5.1 %) representing 16 endemic and 1 introduced host species and were detected from 9 of 11 localities (Figure 3b; see Appendix I). Prevalence varied among taxa (range 0.6 % to 36.4 %) and localities (χ^2 = 43.43, *d.f.* = 10, p < 0.001). Prevalence was significantly higher than average on Mount Latimojong (11.2 %, residuals = 4.23, p = 0.0005) and Mount Gandang Dewata (10.5 %, residuals = 3.44, p = 0.012). Infections were not significantly different than average for Malili (0.0 %, residuals = -1.56, p = 1.00), Mount Ambang (0.0 %, residuals = -2.12, p = 0.74), Mount Balease (1.4 %, residuals = -1.37, p = 1.00), Mount Bawakaraeng (4.5 %, residuals = -0.42, p = 1.00), Mount Bonggolosapi (2.1 %, residuals = -2.21, p = 0.595), Mount Buliohuto (8.5 %, residuals = 1.25, p = 1.00), Mount Dako (3.5 %, residuals = -1.44, p = 1.00), Mount Katopasa (2.9 %, residuals = -1.29, p = 1.00) or Mount Torompupu (4.5 %, residuals = -0.21, p = 1.00).

For Lewisi clade infections, prevalence varied significantly among elevation bins (χ^2 = 21.00, *d.f.* = 5, p = 0.0008; Figure 4b; see Appendix I), but only elevation bin V (2,001 to 2,500 m) showed a significant increase in prevalence (8.7 %, residuals = 3.45, p = 0.007). There were no significant differences for bins I (< 500 m, 2.4 %, residuals = -2.67, p = 0.092), II (501-1,000 m, 3.3 %, residuals = -1.23, p = 1.00), III (1,001-1,500 m, 3.9 %, residuals = -0.82, p = 1.00), IV (1,501-2,000 m, 4.9 %, residuals = -0.19, p = 1.00), or VI (> 2,500 m, 9.2 %, residuals = 2.02, p = 0.52).

Table 4. Orders of Kinetoplastea detected in rodents and shrews of Sulawesi with details of their infections.

Order	Clade, Genus or species	Number infections	% of all infections	BLAST similarity	Rodent hosts (*n*)	Shrew hosts (*n*)	Number localities	Elevation bin range (masl)
Neobodonida		**7**	**1.2%**					
	Neobodo curvifilus	1	0.2%	100%	*Bunomys chrysocomus*		1	501-1,000
	Neobodo cf. designis	1	0.2%	88%		*Crocidura parva*	1	1,501-2,000
	Dimastigella sp.	4	0.7%	96-98%	*Frateromys fratrorum* (2), *Crunomys musschenbroekii* (1)	*Crocidura parva*	3	1,001-2,000
	Genus unidentified	1	0.2%	84-86%	*Frateromys fratrorum*		1	1,001-1,500
Eubodonida		**9**	**1.6%**					
	Parabodo cf. caudatus	9	1.6%	91-99%	*Rattus mollicomulus* (2), *B. penitus* (1), *Crunomys hellwaldii* (1), *C. musschenbroekii* (2), *Rattus facetus* (1)	*Crocidura australis* (1), *C. lea* (1)	4	501-3,000
Trypanosomatida		**549**	**97.0%**					
	Blastocrithidia sp.	1	0.2%	100%		*Crocidura* sp.	1	< 500
	Blechomonas sp.	1	0.2%	98%	*Rattus mollicomulus*		1	1,501-2,000
	Herpetomonas sp.	1	0.2%	99%	*Rattus hoffmanni*		1	501-1,000
	Kentomonas sp.	1	0.2%	97%	*Bunomys andrewsi*		1	< 500
	Genus unidentified	1	0.2%	98-99%	*Taeromys dominator*		1	< 500
	Trypanosoma	**544**	**96.3%**					
	Trypanosoma conorhini	1	0.2%	99%	*Bunomys andrewsi*		1	< 500
	Cruzi clade (*terrestris*-like)	11	1.9%	97-98%	*Crunomys hellwaldii* (10), *Taeromys celebensis* (1)		3	< 500
	Lewisi Clade sp.	90	15.9%	98-100%	17 species (see Table 3)		9	< 500-3,000
	Theileri clade (*T. cyclops*)	442	78.2%	97-99%	26 species (see Table 3)	15 species (see Table 3)	10	< 500-3,000

Cruzi clade (Terrestris-like) infections were detected in 11 individuals representing 2 endemic murid host species (1 *Taeromys celebensis* and 10 *Crunomys hellwaldii*) They were detected from 3 of 11 localities with all infections occurring in disturbed habitat in elevation bin I (< 500 m; Supplementary Material S1).*Trypanosoma conorhini* was detected in a single *Bunomys andrewsi* sample (NMV Z56762) from Mount Katopasa in disturbed habitat in elevation bin I (< 500 m; Supplementary Material S1).

Discussion

The continued detection of zoonotic diseases in human populations has fuelled investment in global wildlife disease surveillance aimed at identifying zoonotic pathogens with potential as emerging infectious diseases in humans (http://www.ceropath.org/; PREDICT Consortium 2020; Blasdell *et al.* 2022). Most of this work has focused on urban and rural habitats, under the expectation that the greatest risk for zoonotic diseases occurs where human-wildlife interactions are most frequent (Bordes et al. 2013; Blasdell et al. 2019, 2022). However, these habitats support a depauperate host community and much less surveillance occurs in the most biodiverse and intact ecosystems where potential zoonoses may be most prevalent and diverse. Our results with trypanosomes reinforce the notion that the zoonotic risk associated with mammal-borne parasites in tropical Southeast Asia (*e. g.* Blasdell et al. 2019, 2022) warrants surveillance beyond the edges of human settlement. This expanded effort will be essential to gain a comprehensive understanding of reservoir hosts, disease interactions and transmission risks.

Our results from screening for trypanosome infections in rodents and shrews offers a wildlife disease model of infection prevalence extending from the edge of human settlement into intact forest habitats on the biodiverse and densely populated, tropical island of Sulawesi, Indonesia. Trypanosome infections were dominated by one native species (Theileri clade (*T. cyclops*)) and one introduced species (Lewisi clade). We detected the native trypanosome in both rodents and shrews and previously reported it from bats (Mursyid *et al.* 2023) indicating that it infects a wide range of mammalian species. In contrast, the introduced species infected only rodents of the family Muridae. Both the native and introduced trypanosomes were detected in all elevation bins, extending from village edges to intact high-elevation forest. However, infections by both trypanosome clades reached their highest prevalence above 2,000 m elevation in the most intact forest habitats away from human habitation. The high prevalence of introduced Lewisi clade trypanosomes within intact forests and their infection of endemic host species associated with high elevation, primary forest (*e. g. Tateomys macrocercus*) reveals the capacity of introduced parasites to leave human settlements and penetrate biodiverse ecosystems. The detection of both trypanosome clades in host species with ranges that span all elevations and occur in both intact and disturbed habitats (*e. g. Crunomys musschenbroekii*, *Rattus hoffmanni*) reveals the capacity for interactions with a larger number of other host species and that may increase the probability of spillover to reservoir hosts and humans (Bordes et al. 2013, 2015). If trypanosome infections are indicative of zoonotic diseases, they suggest that novel disease interactions among native and introduced pathogens may be most prevalent in the most biodiverse habitats. These

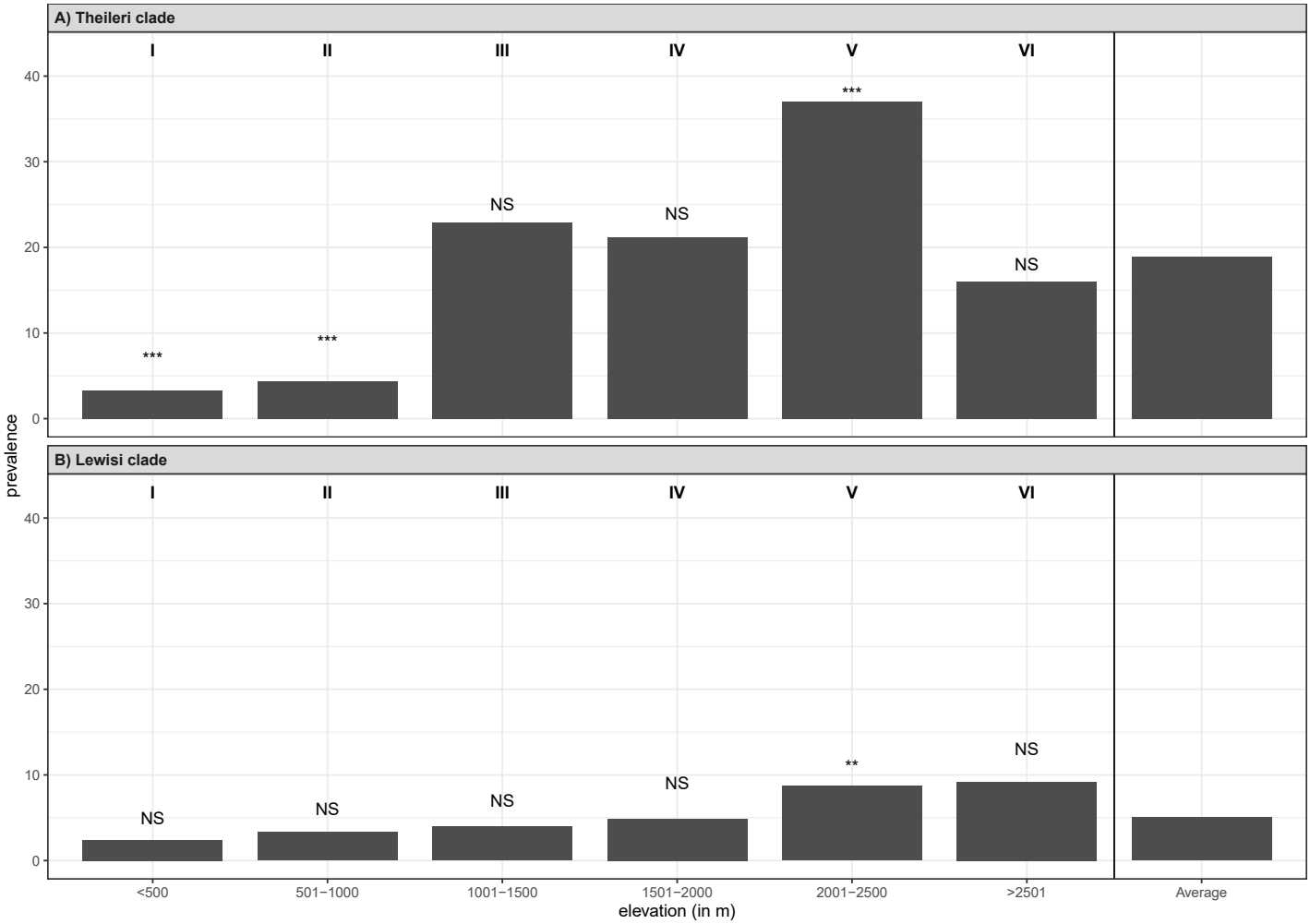

Figure 4. Prevalence of (A) Theileri clade (*T. cyclops*) and (B) Lewisi clade infections in rodents and shrews by elevation bin combining all localities. Roman numerals represent each elevation bin. The average prevalence for all bins is included on the right. Symbols above the bars indicate significance of prevalence by elevation bin compared to the average for that clade ('NS' = not significant, '*' = p = 0.01 – 0.05, '**' = p = 0.001 – 0.01, '***' = < 0.001).

habitats beyond, but connected to, human settlement are likely to host a rich assemblage of zoonotic diseases with the potential to impact native wildlife health and lead to novel wildlife-human transmission events with unknown epidemiological effects.

Identifying zoonotic diseases from species inhabiting the most biodiverse and intact habitats will require leveraging all available samples and engaging with biologists collecting samples in remote conditions. Natural history collections house specimens from the breadth of global biodiversity that could be leveraged to a greater extent in wildlife pathogen research and surveillance (DiEuliis *et al.* 2016; Dunnum *et al.* 2017; Bell *et al.* 2018; Schindel and Cook 2018; Colella et al. 2021). Globally, more than 3 billion museum specimens, spanning centuries of collecting, provide unparalleled access to the historical context lacking in most disease ecology research (Harmon *et al.* 2019; Schmitt *et al.* 2019; Cook *et al.* 2020). They have revealed the origins of diseases (*e. g.* 1918 Spanish Influenza; Taubenberger *et al.* 2007), detected the invasion of parasites (*e. g. Trypanosoma lewisi* on Christmas Island; Wyatt *et al.* 2008), identified the source of disease outbreaks (*e. g.* Sin Nombre Virus; Yates *et al.* 2002), and tracked global trends in parasitism (Wood *et al.* 2023a, 2023b). These specimens retain evidence of infections that have been critical to identifying the geographic and temporal origins of contemporary wildlife diseases such as white-nosed syndrome in bats (Campana *et al.* 2017), retroviruses in koalas (Ávila-Arcos *et al.* 2013), and chytrid fungus in amphibians (Cheng *et al.* 2011; Byrne *et al.* 2019). By preserving specimens before, during and after disease outbreaks, they provide insight into the evolutionary responses of hosts (Foster *et al.* 2007; Mikheyev *et al.* 2015; Cook *et al.* 2017), vectors (White *et al.* 2011; Pelissie *et al.* 2018; Korlević *et al.* 2021; Meireles *et al.* 2023), and pathogens (Yates *et al.* 2002; Tsangaras and Greenwood 2012, 2019; Ávila-Arcos *et al.* 2013; Nishimura *et al.* 2022). Holistic specimens preserve the phenotypic evidence of disease on the health and pathology of animals and their trends over time (Rothschild and Panza 2005; Lorch *et al.* 2021). This notion of the extended phenotype (Webster 2017) revealed by new technologies and new studies of holistic specimens preserved in natural history collections will continue to increase their value to wildlife disease research.

The taxonomically rich, geographically widespread, and temporally deep sampling of natural history collections includes an increasing number of cryogenically frozen tissues, and other material specimens suitable for wildlife disease surveillance. However, a culture of using and growing collections has not been adopted by most global wildlife surveillance projects with few samples deposited in natural history collections and few samples requested for use (Kelly *et al.* 2020; Colella et al. 2021; Thompson *et al.* 2021). Our study exemplifies the scope of samples from biodiverse habitats being acquired by natural history collections that could be leveraged by wildlife disease research. In this study, thousands of ethanol-preserved tissue samples used to screen trypanosomes were collected along with holistic voucher specimens and other tissue specimens preserving DNA and RNA and housed in liquid nitrogen that are amenable to further screening of pathogens including bacteria, viruses, and protozoa. Like many collections of this nature, most species sampled in this study have never been screened for wildlife pathogens and represent the vast discovery awaiting collaboration between natural history collections focused on the breadth of biodiversity and veterinary pathologists focused on zoonotic disease surveillance.

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Appendix I

Prevalence of (A) Theileri clade (*T. cyclops*) infections and (B) Lewisi clade infections of rodents and shrews of Sulawesi by locality, habitat type and elevation bin as detected by PCR. Elevation bins for each locality are shaded to indicate disturbed habitat (dark grey), intact habitat (light grey), mixed habitat (light grey with black box). For mixed habitat bin, richness is separated for disturbed (left of forward slash) and intact (right of forward slash) based on individual traplines. Available but unsampled elevation bins at each locality are shaded without numbers.

A. Theileri clade (*T. cyclops*)

Locality	Infected Disturbed (prevalence)	Infected Intact (prevalence)	Infected Total (prevalence)	Elevation bin I < 500 m	II 500- 1,000 m	III 1,001-1,500 m	IV 1,501-2,000 m	V 2,001-2,500 m	VI > 2,500 m
Malili	6 (12.77%)	NA	6 (12.77%)	6					
Ambang	NA	6 (6.67%)	6 (6.67%)			3	3		
Balease	NA	5 (7.04%)	5 (7.04%)		5	0			
Bawakaraeng	0 (0.00%)	38 (12.71%)	38 (10.89%)				25	13	0
Bonggolosapi	0 (0.00%)	1 (0.41%)	1 (0.39%)	0/0	0	1			
Buliohuto	0 (0.00%)	0 (0.00%)	0 (0.00%)	0	0	0			
Dako	6 (5.88%)	105 (25.36%)	111 (25.51%)	0	6	26	75	4	
Gandang Dewata	1 (6.25%)	45 (22.96%)	46 (21.70%)	0	1		39		6
Katopasa	6 (8.70%)	69 (42.33%)	75 (32.33%)	6	0	42	12	8	7
Latimojong	3 (30.00%)	138 (44.66%)	141 (44.20%)		0	3	0	132	6
Torompupu	1 (25.00%)	12 (7.10%)	13 (7.51%)	1	1		6	5	
Total	**23 (7.23%)**	**419 (20.77%)**	**443 (18.96%)**	**13**	**13**	**75**	**160**	**162**	**19**
				3.32%	4.36%	22.66%	21.14%	36.99%	15.97%
			Sample size	392	298	331	757	438	119

B. Lewisi clade

Locality	Infected Disturbed (prevalence)	Infected Intact (prevalence)	Infected Total (prevalence)	Elevation bin I < 500 m	II 500- 1,000 m	III 1,001-1,500 m	IV 1,501-2,000 m	V 2,001-2,500 m	VI > 2,500 m
Malili	0 (0.00%)	NA	0 (0.00%)	0					
Ambang	NA	0 (0.00%)	0 (0.00%)			0	0		
Balease	NA	1 (1.43%)	1 (1.43%)		1	0			
Bawakaraeng	6 (12.24%)	5 (2.55%)	11 (4.49%)				2	3	6
Bonggolosapi	0 (0.00%)	5 (2.12%)	5 (2.06%)	5/0	0	0			
Buliohuto	1 (12.50%)	5 (7.94%)	6 (8.45%)	1	4	1			
Dako	5 (5.75%)	7 (2.70%)	12 (3.47%)	3	2	2	5	0	
Gandang Dewata	0 (0.00%)	21 (11.29%)	21 (10.45%)	0	0		17		4
Katopasa	0 (0.00%)	5 (4.67%)	5 (2.92%)	0	0	5	0	0	0
Latimojong	0 (0.00%)	26 (11.40%)	26 (11.16%)		0	0	0	26	0
Torompupu	0 (0.00%)	3 (4.76%)	3 (4.48%)	0	0	1	1	1	
Total	**12 (4.2%)**	**78 (5.22%)**	**90 (5.06%)**	**4**	**7**	**9**	**25**	**30**	**10**
				1.06%	3.32%	3.95%	4.90%	8.72%	9.17%
			Sample size	377	211	228	510	344	109

Supplementary material

Supplementary Material S1. List of samples screened in this study with PCR results, Genbank accession numbers, additional information on tissue samples collected.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6154/1476

Supplementary Material S2. Nested trypanosome-generic primers targeting the 18S rDNA locus used in this study.

https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6154/1477

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Influence of late Holocene climate and wildfire on mammalian community composition in the northern Rocky Mountains (USA)

M. Allison Stegner[1]* and Elizabeth A. Hadly[1, 2]

[1]Department of Biology. Stanford University, 371 Jane Stanford Way, 94305-5020, Stanford, California, USA. E-mail: astegner@stanford.edu; (MAS; Current address: Southwestern Biological Science Center, U.S. Geological Survey. Email: mstegner@usgs.gov).
[2]Department of Earth Systems Science. Stanford University, 473 Via Ortega, 94305-5020, Stanford, California, USA. Email: hadly@stanford.edu (EAH).
* Corresponding author: https://orcid.org/0000-0002-1412-8203.

Over the last half century, the Rocky Mountains have experienced increasing temperatures, more frequent droughts, and remarkable increases in wildfire: trends that are expected to continue. While the consequences of ongoing climate and fire regime change for this region are uncertain, previous research suggests that the combination of more frequent fire with changing climate may lead to abrupt changes in vegetation, causing downstream effects including altering mammal communities. Small mammals, in particular, are more habitat-specific and less able to move great distances in response to habitat disturbance. Reconstructing how these ecosystems have responded to climate and fire regime change in the past can reveal underlying dynamics that enable us to anticipate how they will react to future changes. Although there has been substantial research on fire-climate-vegetation relationships, the long-term impacts of fire on small mammal communities are not well characterized. Here, we use mammalian fossils from Waterfall Locality, a fossil packrat midden in northeastern Yellowstone National Park spanning ~3,400 to 250 calendar years before present (cal YBP), to reconstruct mammal diversity (species richness, evenness, and relative abundance) through time and to explore whether changes in diversity and community composition were related to climate and fire regime. We evaluate reconstructed wildfire activity from sedimentary charcoal as well as seven modeled climate variables (mean annual temperature, minimum winter temperature, maximum summer temperature, temperature seasonality, mean annual precipitation, mean summer precipitation, and precipitation seasonality). We find evidence that both summer precipitation and wildfire contributed to small mammal community turnover. Higher summer precipitation was associated with higher proportions of closed-habitat mammals and lower proportions of open habitat mammals. Elevated levels of wildfire activity near the site from ~2,200 – 1,800 cal YBP likely also contributed to this change in closed- versus open-habitat mammals around 1,600 cal YBP. Waterfall Locality represents a ~3,400-year record of mammal diversity in lower montane forests of the northern Rockies, analyses of which provide context for predicting future changes to the mammal community in this region.

Durante el último medio siglo, las Montañas Rocosas han experimentado temperaturas crecientes, sequías más frecuentes y aumentos notables de incendios forestales: tendencias que se espera que continúen. Si bien las consecuencias del actual cambio climático y del régimen de incendios para esta región son inciertas, investigaciones anteriores sugieren que la combinación de incendios más frecuentes con un clima cambiante puede provocar cambios abruptos en la vegetación, causando efectos aguas abajo, incluida la alteración de las comunidades de mamíferos. Los mamíferos pequeños, en particular, son más específicos de su hábitat y menos capaces de moverse grandes distancias en respuesta a la alteración del hábitat. Reconstruir cómo estos ecosistemas han respondido al cambio climático y al cambio del régimen de incendios en el pasado puede revelar dinámicas subyacentes que nos permitan anticipar cómo reaccionarán ante cambios futuros. Aunque se han realizado importantes investigaciones sobre las relaciones entre el fuego, el clima y la vegetación, los impactos a largo plazo del fuego en las comunidades de pequeños mamíferos no están bien caracterizados. Aquí, utilizamos fósiles de mamíferos de Waterfall Locality, un basurero de ratas fósiles en el noreste del Parque Nacional de Yellowstone que abarca aproximadamente 3400 a 250 años calendario antes del presente (cal YBP), para reconstruir la diversidad de mamíferos (riqueza de especies, uniformidad y abundancia relativa) a través de tiempo y explorar si los cambios en la diversidad y la composición de la comunidad estaban relacionados con el clima y el régimen de incendios. Evaluamos siete variables climáticas modeladas (temperatura media anual, temperatura mínima en invierno, temperatura máxima en verano, estacionalidad de la temperatura, precipitación media anual, precipitación media en verano y estacionalidad de la precipitación), así como la actividad de incendios forestales reconstruida a partir de carbón sedimentario. Encontramos evidencia de que tanto las precipitaciones de verano como los incendios forestales contribuyeron al recambio de las comunidades de pequeños mamíferos. Las mayores precipitaciones de verano se asociaron con mayores proporciones de mamíferos de ambientes cerrados y proporciones más bajas de mamíferos de ambientes abiertos. Los niveles elevados de actividad de incendios forestales cerca del sitio de ~2200 a 1800 cal YBP probablemente también contribuyeron a esto en mamíferos de ambiente cerrado versus abierto alrededor de 1600 cal YBP. La localidad de Waterfall representa un registro de ~3400 años de diversidad de mamíferos en los bosques montanos bajos del norte de las Montañas Rocosas y proporciona un contexto para cambios futuros en la comunidad de mamíferos en esta región.

Keywords: Holocene; northern Rocky Mountains; packrats; small mammals; vegetation change; wildfire; Yellowstone National Park.

Introduction

The climate of the northern Rocky Mountains is projected to exceed historical ranges of variability within this century (Meehl *et al.* 2007), leading to increases in fire frequency (Westerling *et al.* 2011) with unclear ramifications for Rocky Mountain forested ecosystems. Wildfires in the northern Rockies increased 889 % in frequency and 2,966 % in area burned from 1973 to 1983 and 2003 to 2012 (Westerling 2016). Wildfire activity is strongly tied to climate in western North America: increased fire risk is correlated with higher spring and summer temperatures and earlier snowmelt (Westerling *et al.* 2006). Reconstructing how these ecosystems have responded to climate change and increased fire in the past can reveal underlying dynamics that enable us to anticipate how they will react to future changes.

Forests of the northern Rockies are adapted to recurrent stand-replacing wildfires on the scale of centuries to millennia, but changes in fire frequency and severity in combination with changing temperature and/or precipitation can lead to abrupt shifts in vegetation composition (Sánchez Goñi 2017; Hansen *et al.* 2018; Hansen and Turner 2019). Globally, recent fires paired with rising temperatures have already caused abrupt vegetation changes in montane, subalpine, and boreal conifer forests (Johnstone and Chapin 2003; Wirth *et al.* 2008; Johnstone *et al.* 2010; Savage *et al.* 2013; Hansen *et al.* 2016; Coop *et al.* 2020; Hill *et al.* 2023). Although the northern Rockies have been an important region for the study of fire-climate-vegetation research in the paleontological (*e. g.*, Millspaugh 1997; Huerta *et al.* 2009; Higuera *et al.* 2010; Power *et al.* 2011; Krause and Whitlock 2017; Iglesias *et al.* 2018; Stegner *et al.* 2019) and neontological record (*e. g.*, Turner *et al.* 1997, 1999; Schoennagel *et al.* 2004; Donato *et al.* 2016; Hansen and Turner 2019), we know far less about recent, long-term (multi-decadal to millennial) impacts of fire regime on vertebrates, and especially small mammals (Lyon *et al.* 2000; Culhane *et al.* 2022) that make up the majority of mammal diversity (Damuth 1987).

Because of the rarity of and temporal mismatch between vertebrate fossil deposits and the paleoclimatic/paleofire records, there have been no studies attempting to directly link vertebrate community change with past fire dynamics in the northern Rockies. Here, we report on mammal community change in Waterfall Locality, a packrat midden spanning ~3,400 to 250 years before present (YBP) in northeastern Yellowstone National Park, USA. Evidence of fires at Waterfall Locality is provided by abundant charcoal in the midden and by fire-related debris flows throughout the watershed (Meyer *et al.* 1995a). Specifically, we address the question: did mammal diversity change over the last ~3,400 years, and, if so, are those changes related to changes in temperature, precipitation, and/or wildfire? Since the end of the Pleistocene, fire frequency in the vicinity of Waterfall Locality reached its highest level in the last 2000 years (Millspaugh 1997), so the period spanned by the deposits at this locality encompass important climatic and fire regime changes which herald further changes expected in the coming decades. In particular, Waterfall Locality captures the Medieval Climatic Anomaly (MCA; 950 AD to 1250 AD; Mann *et al.* 2009), which includes decadal periods of high temperature comparable to present day, though of lower intensity and higher overall variability (Heeter *et al.* 2021). Additionally, while packrats are well-known vectors of fossilization in arid regions of North America, packrat-accumulated sites have received less attention in montane environments (But see: Porcupine Cave, Colorado Rocky Mountains [Barnosky 2004]; Lamar Cave, Wyoming Rocky Mountains [Hadly 1996]; Signature, Haystack, and Cement Creek Caves, Colorado Rocky Mountains [McLean and Emslie 2012; McLean *et al.* 2014; Emslie *et al.* 2019]; Bear Den Cave, Sierra Nevada Mountains [Mead *et al.* 2006]) and thus Waterfall Locality contributes to our understanding of montane ecosystems, which are widespread and biologically important in North America.

Materials and methods

Site Description. Waterfall Locality is located along an unnamed drainage off Soda Butte Creek in northeastern Yellowstone National Park, USA, ~10 km southwest of Silver Gate, Montana, at an elevation of 2,260 m (latitude 44.96120, longitude -110.06473; Figure 1). The vegetation around Waterfall Locality today is a mosaic of Douglas-fir forest/woodland, spruce-fir forest/woodland, lodgepole pine forest, subalpine woodland, montane meadows, sagebrush steppe, and riparian communities (LANDFIRE 2022; Figure 1C and D). Spruce-fir forest immediately surrounds the deposit. Northeastern Yellowstone receives most of its precipitation in spring and summer, and winters are fairly dry (Whitlock and Bartlein 1993); average annual precipitation in the area is 80 cm (Licciardi and Pierce 2018). The site is a packrat midden accumulation in a fissure beneath a dolomite cliff, which was discovered in 1990 when a flood in the drainage eroded the front of the midden and exposed the accumulation. The maximum dimensions of the deposit prior to excavation were approximately 2.2 m high, 3.0 m wide, and 3.0 m deep (Figure 2a). Immediately upstream, the slope becomes very steep with sparse vegetation through treeline (~2,700 m) up to Abiathar Peak (3,332 m; Figure 1C and D).

Prior to excavation, the surface of the accumulation was covered with packrat scats, conifer needles, dolomite clasts, sticks, and miscellaneous debris. Because the accumulation is situated in a narrow vertical fissure, it is evident that the carnivore scats, raptor pellets, miscellaneous bones and other materials were collected by packrats (*Neotoma cinerea*), and that this site did not function as a carnivore den. Additionally, the bones show evidence of rodent gnawing, but not of carnivore gnawing, and the bones are generally quite small (<3 cm). The few large mammal species present in the assemblage are represented by small specimens likely derived either from carnivore scats or possibly from animal carcasses found near the midden. Previous research

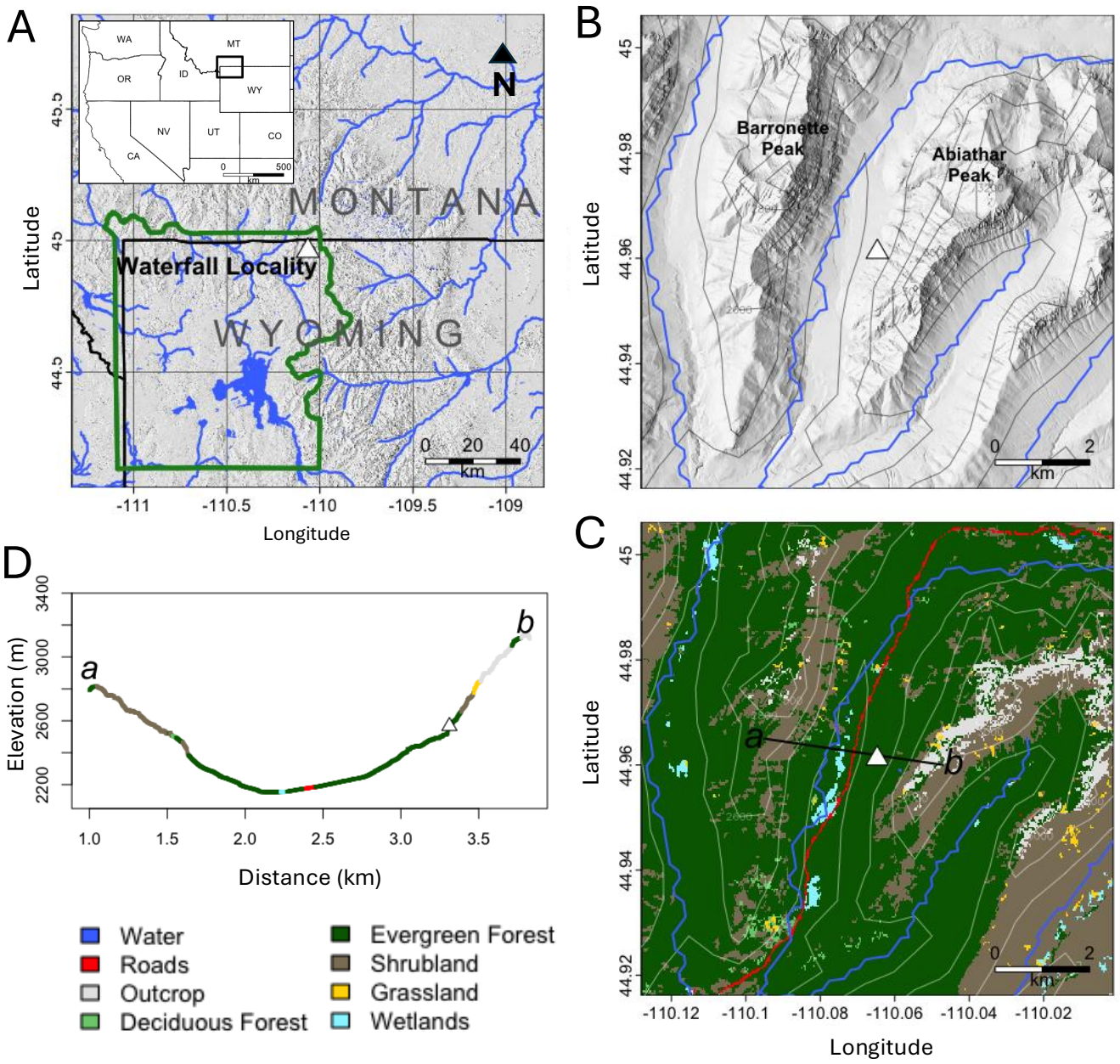

Figure 1. Map of the study area. A) Regional map; green border shows the Yellowstone National Park border; white triangle shows location of Waterfall Locality. B) Elevation map of the immediate vicinity of Waterfall Locality (white triangle), including streams and nearest peaks. C) Vegetation map of the immediate vicinity of Waterfall Locality. Transect a-b is represented in D), vegetation type across elevation gradient as a function of distance from point a to point b. Color scheme matches C). Elevation data are from the National Elevation Dataset at a resolution of 1/3 arcsecond (U.S. Geological Survey 2019), and vegetation cover data are from the National Land Cover Database (Dewitz 2021), both accessed using the R *FedData* package (Bocinsky 2024).

has demonstrated with high confidence (95 %) that the sampling radius for bones recovered from Waterfall Locality is 17.5 km or less (Porder *et al.* 2003).

Excavation. Waterfall Locality was excavated in June and July of 1991 by Elizabeth A. Hadly and a team of field assistants (NPS Permits YELL #00200; YELL #05638). The deposit was interspersed with dolomite roof falls and occasional flooding of the site from the adjacent unnamed creek. These sedimentary events helped to define discrete sections of the midden and facilitated excavation in natural stratigraphic units. Unit numbers from 1 to 19 were assigned from top to bottom for the natural strata. Strata thicker than 10 cm were subdivided into arbitrary units A through C, although these subunits were analytically combined for this study, with the exception of Unit 9A (Supplemental 1). Prior to excavation, talus around the accumulation was cleared and designated as "Undifferentiated." Excavation was done by hand with a trowel, and excavated material was placed in buckets and washed through a series of five stacked screens of decreasing mesh size (4, 8, 16, 20, and 30 squares per square inch). Matrix was dried in the field, bagged, and transported to the lab. During excavation, the deposits collapsed twice, and the collapsed material was collected and designated as "Undifferentiated Units 1-3" and "Undifferentiated above Unit 14." Specimens are deposited with the Heritage and Research Center in Yellowstone National Park.

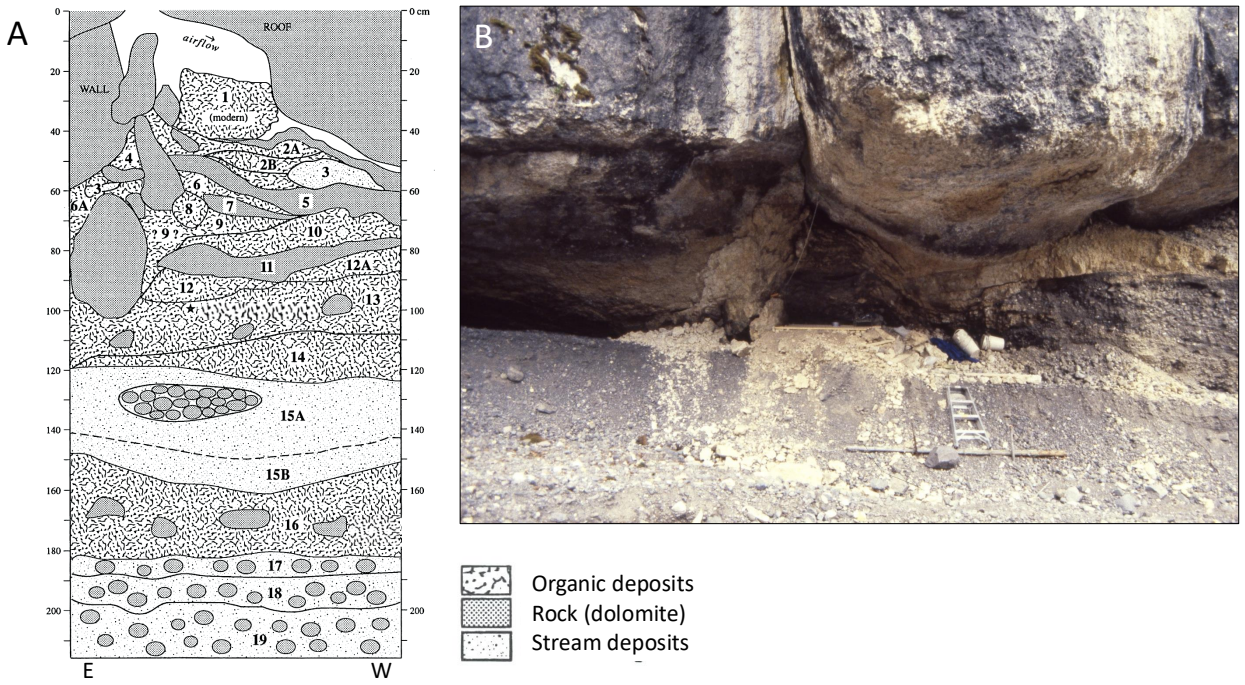

Figure 2. Stratigraphy of Waterfall Locality. A) Stratigraphic section of Waterfall Locality. Numbers refer to excavation units; see Supplemental 1 for detailed lithologic description. B) Photograph of the deposit during excavation.

Chronology. To develop a chronology, we radiocarbon-dated 8 bone and 13 charcoal samples (Table 1). Samples were selected to span the deposits, and to target particularly fossiliferous units. We dated multiple samples for some units to better understand time-averaging within units and through the deposit. Of the fossil-bearing units, 1, 4, and 15 were not directly dated. Unit 1 is considered modern. When possible, we paired bone and charcoal dates to assess whether there were systematic differences in age estimates from these sources. All samples were dated using accelerator mass spectrometry (AMS), with the exception of two large charcoal pieces (AA-7962 and AA-7963, Table 1). We rejected one date (CAMS-83292) because it was ~40,000 years older than the second oldest date, also from level 16, and is unlikely to be accurate. Note that this area was covered by glaciers until at least 14,900 YBP (Licciardi and Pierce 2018).

Using the *Bchron* package (Haslett and Parnell 2008) in R (R Core Team 2021), radiocarbon dates were calibrated using the IntCal20 calibration curve (Reimer *et al.* 2020). We generated a Bayesian age model using the accepted radiocarbon dates and the top of the deposit as chronologic controls (Figure 3).

To assess the effect of charcoal versus bone samples on estimates of the age of each unit, we created three Bayesian age models using just charcoal, just bone, or both charcoal and bone dates (including the age of the top of the deposit as the year of excavation in all cases). We then examined overlap of the 95% confidence intervals for all three models (Supplemental 3).

Taxonomic Identification. Fossil material was removed from the sieved bulk matrix by hand or with forceps and sorted taxonomically. Mammalian remains were identified to the lowest taxonomic level possible, typically genus or species. For rodents, lagomorphs, and insectivores, only craniodental material was used for identification; all other mammals (carnivores and artiodactyls) were identified based on both cranial and post-cranial diagnostic specimens. Comparative material from the University of California Museum of Vertebrate Zoology and from the Hadly Morphology Laboratory (Stanford University, California, USA) comparative collections (including vertebrate specimens collected within Yellowstone National Park; permits YELL #00200; YELL #05638) were used to aid identifications. Plant, mollusk, insect, and bird material were not identified, and no fish, reptile, or amphibian remains were recovered at the site.

Analysis of diversity change. We calculated the number of identified specimens (NISP) and minimum number of individuals (MNI) for each taxon and traced changes in diversity through time using taxonomic richness, relative abundance, and evenness. We excluded large-bodied mammals and carnivores from diversity analyses because they were likely introduced to the deposit via different taphonomic pathways than small mammals, which would have been embedded in carnivore scats or raptor pellets (Hadly 1999). To account for differences in sample size, we standardized species richness using Shareholder Quorum Subsampling (SQS) at a coverage level of 0.8 (Alroy 2010). In contrast to rarefaction which fixes sample size, SQS fixes coverage, the proportion of the entire frequency distribution represented by the species in the resample. SQS-estimated richness values are lower than raw richness but are linearly proportional to one another, and species-rich sites are not penalized as they are in rarefaction (Alroy 2010). Evenness

Table 1. Waterfall Locality radiocarbon ages.

Lab Number	Excavation Unit	^{14}C Age	^{14}C Error	Material	Calibrated YBP [a]
CAMS-83274	2A	355	40	bone	399 (315-492)
CAMS-83282	2A	580	40	charcoal	598 (529-645)
CAMS-83275	2B	645	40	*Neotoma* mandible	604 (554-666)
CAMS-83283	2B	815	40	charcoal	718 (676-794)
CAMS-83276	6	1590	60	bone	1466 (1355-1610)
CAMS-83284	6	1865	50	charcoal	1776 (1637-1908)
CAMS-83277	8	2215	40	*Neotoma* mandible	2225 (2115-2329)
CAMS-83278	9	1645	45	*Neotoma* mandible	1522 (1411-1683)
CAMS-83287	9	2070	40	charcoal	2031 (1920-2142)
CAMS-83286	9	2555	35	charcoal	2640 (2504-2746)
CAMS-83285	9A	1050	40	charcoal	953 (835-1053)
CAMS-83288	10	1800	40	charcoal	1694 (1589-1812)
CAMS-83279	10	2460	35	bone	2549 (2372-2703)
CAMS-83289	12	2365	40	charcoal	2401 (2331-2665)
CAMS-83280	13	2495	40	bone	2581 (2393-2720)
CAMS-83290	13	2840	40	charcoal	2948 (2852-3095)
AA-7962	13	2895	50	charcoal	3033 (2884-3179)
CAMS-83281	14	2660	40	*Neotoma* mandible	2772 (2736-2850)
CAMS-83291	14	3020	40	charcoal	3219 (3079-3341)
AA-7963	16	3020	65	charcoal	3212 (3005-3362)
CAMS-83292	16	39600	2100	charcoal	43264 (40465-46256)[b]

[a] Radiocarbon ages calibrated using the IntCal20 calibration curve (Reimer *et al.* 2020) in the R *Bchron* package (Haslett and Parnell 2008).
[b] Sample excluded from further analyses.

was calculated using Hurlbert's probability of interspecific encounter (PIE; Hurlbert 1971). We computed variance in PIE following Davis (2005) with a sample size of 20 and 1000 iterations. Because time bins are likely to be serially autocorrelated, we assessed significant differences in PIE among time bins by comparing overlap in variance (Blois *et al.* 2010; Stegner 2016). We calculated relative abundance from the NISP. Previous research has shown that NISP is a less-biased indicator of the relative importance of a taxon as compared to MNI (Blois *et al.* 2010; Grayson 1978).

To understand the role of shifting environments in driving diversity change at Waterfall Locality, we classified taxa as associated with closed habitats (*i. e.*, forest), open habitats (grassland, riparian, etc.), or no preference (Supplemental 4). Information about habitat preference was assembled from Foresman (2001), Streubel (1995), and Zeveloff (1988) and from personal small-mammal trapping experience in the montane western USA. We compared relative abundance of open versus closed taxa through time and calculated the 95 % confidence intervals around each relative abundance estimate using Goodman's (1965) simultaneous confidence intervals (Calede *et al.* 2011; McHorse *et al.* 2012; Stegner 2016), as calculated in the R *DescTools* package (Signorelli *et al.* 2015).

Changes in sampling vectors. Raptor pellets and mammalian carnivore scats are a common component of packrat middens and are the primary source of bone in these deposits (Hadly 1999). Because scats and pellets from either nocturnal or diurnal predators may be represented in packrat middens, changes in the relative abundance of nocturnal and diurnal prey species recovered from the deposit are indicative of a change in predator composition, and therefore a change in taphonomy. This is important to understand because shifts between predominantly nocturnal or diurnal taxa in the deposit could be mistaken for community turnover on the landscape. We analyzed the proportion of nocturnal versus diurnal taxa found in Waterfall Locality through time. We initially classified small mammals as strictly diurnal, mostly diurnal, no preference, mostly nocturnal, or strictly nocturnal. We then quantified relative abundance of mostly/strictly diurnal and mostly/strictly nocturnal and calculated Goodman's simultaneous confidence intervals.

Influence of climate on mammal community composition. We tested for a relationship between four biodiversity metrics—taxonomic richness (SQS), evenness (PIE), proportion of closed habitat taxa, and proportion of open habitat taxa—and seven measures of past climate. Climate variables were mean annual temperature, minimum winter temperature, maximum summer temperature, temperature seasonality, mean annual precipitation, mean summer precipitation, and precipitation seasonality. We used PaleoView (Fordham *et al.* 2017) to download modeled climate time series data averaged over 30-year intervals with 20-year interval steps, for the finest latitude/longitude grid cell (latitude: 42.5 to 45.0° N, longitude: 112.5 to 110.0° W)

that included Waterfall Locality. We estimated the value of each climate variable during the time when each excavation level was deposited. To account for the age range of each excavation unit, we resampled the ages for each level from the Bayesian age model posteriors, which represent the iterative model runs for the age model. We matched each of the 1000 age estimations for each level to the closest age in the climate time series to calculate a range of possible climate values for each level.

To correlate each climate variable to each of the biodiversity measures, we sampled the distribution of possible climate values for each level, calculated the Pearson's product moment correlation between the biodiversity metric and climate through time, then reiterated this process 1000 times. This generated a distribution of observed Pearson's test statistics. We compared this to a distribution of null Pearson's test statistics generated by permutation. We used student's t-tests to determine if the distributions of observed and null test statistic distributions were significantly different from one another.

In *Thomomys talpoides*, the pocket gopher found at Waterfall Locality, mandibular diastema length has been shown to be an ecophenotypically controlled trait related to nutritional quality and elevation (Hadly 1997). To assess the elevation from which specimens in Waterfall Locality were derived over time, following Hadly (1997), we measured *T. talpoides* diastema length for comparison to previously published data from modern and fossil *T. talpoides* in the Yellowstone region.

Influence of wildfire on mammal community composition. Millspaugh (1997) estimated fire frequency over the last 2000 years at Slough Creek Pond (~25 km west of Waterfall Locality) to be between 12 and 17 fires per 1000 years, meaning that fires were taking place every ~60 to 80 years on average in the surrounding sagebrush/grassland. The upper Soda Butte Creek drainage where Waterfall Locality is located is in a narrow canyon at a higher elevation and dominated by a mixed forest comprised of spruce (*Picea engelmannii)*/fir *(Pseudotsuga menziesii)*/ lodgepole pine *(Pinus contorta)* forest. Analyses of stand age and fire scars in these forests suggest that fires occur less frequently than in sagebrush/grasslands: on average every 100 to 300 years (Romme and Knight 1982; Romme and Despain 1989).

In northeastern Yellowstone National Park, large fire events over the past 3,500 years were found to be followed by fire-related sedimentation events such as debris flows (Meyer *et al.* 1992; Meyer *et al.* 1995a). Meyer *et al.* (1995a) radiocarbon dated charcoal from debris flows along streams in the Soda Butte Creek basin, which includes Waterfall Locality. Charcoal is produced by wildfires, and it becomes entrained in debris flows when precipitation falls on steep, burned areas which are highly susceptible to erosion and are characteristic of the glacially over-steepened Soda Butte Valley (Meyer *et al.* 1995a). Debris flows are deposited on alluvial fans along streams and rivers and are characterized by poorly sorted muddy sands with abundant charcoal and organic material (Meyer *et al.* 1992). The "old wood" effect—the combined effect of dating long-lived organisms like trees and the long environmental residence time of charcoal—may affect these charcoal dates. The debris flow charcoal data providing a record of past wildfires in the vicinity of Waterfall Locality has a systematic age bias of <80 years, where charcoal ages predate fire events by ~60 to 80 years.

The estimated probability density of fire-related debris flows in upper Soda Butte Creek used summed probability densities of the charcoal radiocarbon dates (Meyer *et al.* 1995a). It has since been shown that summed probability densities of radiocarbon dates conflate age uncertainty with process variation (Carleton and Groucutt 2020), in this case variation in fire frequency. Therefore, to quantify past fire activity, we evaluated the number of charcoal pieces as a proxy for the number of fires through time. Meyer *et al.* (1995a) examined 58 likely or definite debris flows throughout the basin. The authors generally sampled one piece of charcoal per debris flow and noted how confident they were that the material came from a debris flow and instances where an event was sampled multiple times. This charcoal sampling method differs from typical charcoal counting methods in sediment cores (*e. g*. Ejarque *et al*. 2015) and so the commonly applied CharAnalysis method (Higuera *et al.* 2009) for reconstructing past fire events was not possible in this case. We included only one charcoal piece per debris flow, so the dataset used here, which includes 53 charcoal samples, is not biased by over-representation of particular fire events. Instead, we estimated the number of charcoal pieces in hundred-year time bins by sampling the calibrated age distribution of each charcoal sample, tallying the number of charcoal samples in each time bin, and iterating 1,000 times. We calculated the median and 95 % confidence interval across iterations. Number of charcoal pieces per century was then qualitatively compared to relative abundance of closed versus open habitat taxa and compared to the known climatic anomalies such as the Medieval Warm Period.

Results

Excavation and Stratigraphic Interpretation. The deposits are comprised of alternating organic-rich packrat midden material, alluvium, dolomite clasts from roof fall, and flood deposits containing Eocene volcanoclastic-derived gravels and cobbles which were likely deposited over hours to days when the adjacent stream overflowed (Figure 2b; Supplemental 1). Bones of bushy-tailed packrats (*Neotoma cinerea*) are present throughout the assemblage, as are *Neotoma* scat and midden materials, providing a consistent indicator that this species accumulated the organic deposits. Charcoal was found in varying concentrations throughout. Bone preservation at all levels was generally sufficient for identification, although bones were frequently broken. Only one articulated skeleton was found, belonging to a chipmunk (*Tamias* sp.).

Units 3, 5, 7, 11, 17, 18, and 19 represent either roof fall or debris flow deposits and although some bone was recovered in most of these units (Supplemental 2), this was likely due to contamination from units above or below and thus these materials were not included in our analyses.

Chronology. Ages of both bone and charcoal are generally older with increasing depth, although there is time-averaging within stratigraphic units (Table 1, Figure 2). For the seven units where both bone and charcoal were dated, in all but one case (Unit 10) the charcoal dates were older than the bone dates (mean difference = 296 years older). Some difference within the same unit is expected due to time-averaging, but a systematic bias of older charcoal dates suggests the "old wood" effect (*e. g*., Schiffer 1986), deviations which depend on the sources of charcoal. Common trees in the vicinity and particularly upstream of Waterfall Locality today include Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), which can live over 500 years and 250 years respectively, which is on par with the mean difference in age between charcoal and bone samples from the same excavation units. Differences in radiocarbon ages even within individual trees may span centuries (Piovesan *et al*. 2018), suggesting that charcoal from long-lived individual trees may also span hundreds of years in this area.

When we compared Bayesian age models using dates from only charcoal or only bone samples, we found differences between the two models for most of the record (Supplemental 3). The charcoal model was older than the bone model for Units 2 and 13 to 17 (with some overlap confidence intervals), but younger in Units 7, 8, 9A, and 10. Given these differences and the likelihood that the charcoal is older than the bone deposited at each level, for all analyses we used the age model which included only radiocarbon dates on bone chronologic controls (Figure 3).

There was considerable overlap in the estimated ages for Units 8 and 9, and Units 12 and 13, implying rapid deposition. We therefore combined these two pairs of units, creating Units 8-9 and Unit 12-13, for all subsequent analyses.

Taxonomic Identification. Mammalian faunal remains (2,406 identifiable specimens) recovered from Waterfall Locality span approximately 3,400 years and represent 5 orders, 12 families, and at least 26 genera and 29 species (Table 2; Supplemental 2). Small and medium-sized mammals (discussed below in Section 4.4) were the most common taxa found in the deposit. Large-bodied herbivores (*Cervus elaphus*, *Odocoileus hemionus*, *Bison bison*, *Ovis canadensis*, *Erethizon dorsatum*) and carnivores (*Puma concolor*, *Lynx* sp., *Mustela erminea*, *Mustela frenata*, *Neovision vision*) appeared sporadically in the deposits, but were too rare to be analyzed statistically (Table 2, Supplemental 2). Although bird fossils were recovered (not discussed here), the site does not contain other vertebrates such as bats, fish, amphibians, or reptiles.

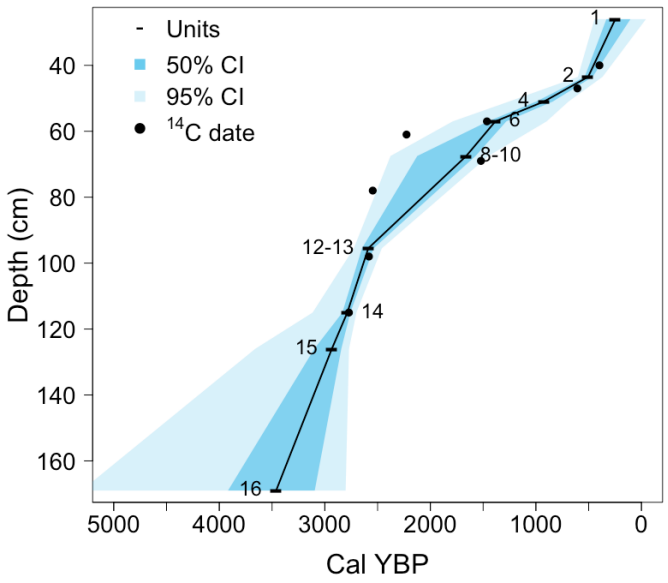

Figure 3. Bayesian age model for Waterfall Locality. Solid blue line is the median age estimate for each excavation unit; light blue shading indicates the 95 % quantiles; dark blue shading indicates the 50 % quantile; black circles show the median age estimate for the radiocarbon dates; black dashes show the mean depth for each excavation level (numbered).

Changes in Small Mammal Diversity. SQS-corrected richness ranged from 3.2 to 5.9, representing an almost 2x increase in richness from the least to the most diverse level (Units 15 and 2, respectively; Table 3). Fluctuations in taxonomic richness were otherwise relatively small across the record. Evenness (PIE) ranged from a minimum of 0.78 (95 % CI = 0.67 – 0.86) in Unit 10, to a maximum of 0.92 (9 5% CI = 0.85 – 0.95) in Unit 8-9, and overlapped for all levels, except that Unit 10 had significantly lower evenness than Unit 9A (and had negligible overlap with Units 16 and 8-9) (Table 3).

Microtus spp., *Urocitellus armatus*, *Neotoma cinerea,* and *Thomomys talpoides* were the most abundant taxa found throughout the record (Figure 4). *Neotoma cinerea* was not found in Unit 10, and *Urocitellus armatus* was missing from Unit 9A, but sample sizes in these units were low (26 and 36 specimens respectively). Leporidae as a group were found throughout, but more taxonomically resolved species—*Lepus* sp., *Lepus* c.f. *L. americanus*, *Sylvilagus* c.f. *S. nuttallii*, and *Ochotona princeps*—occurred sporadically, possibly because lagomorphs are difficult to identify to species- or even genus-level. *Sciuridae, Tamias* spp., *Peromyscus maniculatus*, and *Phenacomys intermedius* were present in relatively low abundances throughout the record.

Early in the record, in Units 16 through 12-13, (3,408 – 2,594 cal YBP, 95% CI = 5,030-2,455 cal YBP), the major components of the small mammal community included *Neotoma cinerea*, *Microtus* spp., *Myodes gapperi, Thomomys talpoides*, *Sorex* sp., leporids, and, in lower abundances, *Urocitellus armatus*, *Callospermophilus lateralis*, *Tamias* spp., *Peromyscus maniculatus*, *Phenacomys intermedius*, and *Zapus princeps* (Figure 4). In Unit 14 (2,784 cal YBP, 95 % CI =

Table 2. Number of Individual Specimens (NISP) and Minimum Number of Individuals (MNI) for taxa found in Waterfall Locality. Units 2A and 2B were combined to form Unit 2; Units 8 and 9 were combined to form Unit 8-9, and Units 12 and 13 were combined to form Unit 12-13. Only data from the excavation units analyzed are included here—full NISP data is available in Supplemental 2.

Order/Family/Genus/Species	1	2	4	6	8-9	9A	10	12-13	14	15	16
Carnivora											
Felidae											
Puma concolor		1 (1)									
Mustelidae											
Mustela erminea				4 (1)							
Mustela frenata		1 (1)						7 (1)			
Neovison vison					1 (1)						
Artiodactyla				1 (1)							
Bovidae											
Ovis canadensis		3 (1)						1 (1)			
Cervidae											
Cervus elaphus	1 (1)			1 (1)							
Odocoileus hemionus	1 (1)	1 (1)			1 (1)			1 (1)			
Eulipotyphla											
Soricidae											
Sorex spp.		4 (1)	1 (1)	3 (1)	38 (3)	5 (1)		7 (1)	14 (2)	2 (1)	
Sorex (*Otisorex*) sp.			4 (1)		5 (1)						32 (1)
Rodentia											
Sciuridae	2 (1)	6 (1)	1 (1)	4 (1)	15 (3)	1 (1)	1 (1)	7 (1)			
Marmota flaviventris	2 (1)	6 (1)	9 (1)		5 (2)			4 (1)			
Urocitellus armatus	21 (3)	40 (4)	15 (2)	20 (3)	87 (7)		6 (1)	130 (7)	8 (2)	2 (1)	9 (1)
Callospermophilus lateralis		10 (1)	1 (1)	3 (1)	21 (3)	5 (1)		23 (3)	1 (1)	2 (2)	
Tamias spp.	1 (1)	5 (1)	4 (1)	13 (2)	16 (3)	1 (1)	1 (1)	6 (2)		5 (1)	6 (1)
Tamiasciurus hudsonicus	2 (1)	5 (1)		1 (1)	10 (2)	1 (1)	5 (1)	18 (3)	1 (1)		
Glaucomys sabrinus		3 (1)		1 (1)	11 (3)			5 (2)	2 (1)		1 (1)
Geomyidae											
Thomomys talpoides	2 (1)	16 (2)	6 (2)	5 (1)	82 (8)	1 (1)		38 (5)	9 (2)	20 (3)	10 (2)
Dipodidae											
Zapus princeps	5 (2)	12 (2)			1 (1)			4 (2)		1 (1)	
Cricetidae											
Myodes gapperi	8 (2)	3 (1)	1 (1)	11 (2)	36 (5)			70 (11)	16 (3)	26 (4)	33 (5)
Microtus spp.	26 (5)	72 (7)	11 (2)	26 (5)	82 (12)	1 (1)	15 (3)	46 (6)	14 (3)	11 (2)	13 (2)
Microtus pennsylvanicus		2 (1)			1 (1)						
Microtus richardsoni		1 (1)		3 (1)				2 (1)			
Phenacomys intermedius	6 (1)	18 (4)	3 (1)	7 (3)	72 (9)		3 (1)	24 (6)	9 (2)	12 (2)	8 (1)
Neotoma cinerea	14 (3)	64 (7)	13 (3)	16 (3)	84 (8)	4 (1)		23 (4)	13 (2)	25 (4)	6 (1)
Peromyscus cf. *P. maniculatus*	6 (2)	12 (3)	3 (2)	12 (2)	66 (14)	1 (1)	3 (1)	16 (4)	7 (2)	5 (1)	6 (3)
Erethizontidae											
Erethizon dorsatum						5 (1)					
Lagomorpha				1 (1)	4 (1)			7 (2)			
Leporidae	3 (1)	46 (3)	5 (1)	3 (1)	74 (5)	4 (1)	2 (1)	57 (4)	14 (2)	14 (2)	1 (1)
Lepus cf. *L. americanus*	1 (1)	1 (1)			5 (4)			16 (3)	1 (1)	1 (1)	2 (1)
Lepus sp.		3 (2)	3 (1)					1 (1)		1 (1)	6 (1)
Sylvilagus cf. *S. nuttallii*		2 (1)			5 (3)	1 (1)		1 (1)			
Ochotonidae											
Ochotona princeps	5 (1)	2 (1)				1 (1)					

Table 3. Richness, evenness, and sample size for each excavation unit. SQS = shareholder quorum subsampling species richness; PIE = Probability of intraspecific encounter species richness; NISP = Number of Individual Specimens.

Analysis Unit	Calendar YBP [95% CI]	Sample size (NISP)	Richness (Raw)	Richness (SQS)	Evenness (PIE) [95% CI]
1	256 [-52 – 446]	104	10	4.01	0.87 [0.78 – 0.93]
2	502 [367 – 603]	333	11	5.94	0.88 [0.79 – 0.94]
4	926 [692 – 1245]	80	10	5.29	0.9 [0.83 – 0.94]
6	1389 [879 – 1919]	129	11	3.62	0.9 [0.82 – 0.94]
8-9	1580 [1237 – 2256]	720	9	4.39	0.92 [0.85 – 0.95]
9A	1627 [1380 – 2308]	26	10	4.33	0.91 [0.87 – 0.93]
10	2417 [2139 – 2585]	36	10	5.01	0.78 [0.67 – 0.86]
12-13	2594 [2455 – 2721]	505	11	5.33	0.89 [0.78 – 0.94]
14	2784 [2705 – 3036]	109	8	3.53	0.9 [0.85 – 0.94]
15	2928 [2765 – 3650]	127	8	3.19	0.87 [0.79 – 0.92]
16	3408 [2796 – 5030]	133	10	5.77	0.86 [0.75 – 0.92]

3,036 – 2,705 cal YBP), *Urocitellus armatus* began to increase, reaching a peak in Unit 12-13 (2,594 cal YBP, 95 % CI = 2,721 – 2,455 cal YBP), and *Glaucomys sabrinus* appeared in low abundance in Units 14 and 12-13. Also in Unit 12-13, we found *Marmota flaviventris.*

There was an overall community shift in Units 10 (2,417 cal YBP, 95 % CI =2,585 – 2,139 cal YBP) and 9A (1,627 cal YBP, 95 % CI = 2,308 – 1,380 cal YBP) as compared to earlier units, though the timing varied somewhat among taxa. Beginning in Unit 10, *Myodes gapperi, Thomomys talpoides*, and *Zapus princeps* disappeared while *Tamiasciurus hudsonicus* and *Microtus* sp. increased (Figure 4). *Callospermophilus lateralis* increased to peak abundance in Unit 9A, simultaneous with an isolated appearance of *Ochotona princeps*. *Tamias* spp., *Peromyscus maniculatus*, *Phenacomys intermedius*, Leporids, and *Sorex* spp. persisted.

In Unit 8-9 (1,580 cal YBP, 95 % CI = 2,256 – 1,237 cal YBP) and above, we saw recovery of some taxa that had been common in Unit 12-13 and earlier (before ~2,600 cal YBP), including *Urocitellus armatus*, *Myodes gapperi,* and *Thomomys talpoides* (Figure 4). *Glaucomys sabrinus* and *Marmota flaviventris* returned in low abundance. However, *Callospermophilus lateralis* and *Tamiasciurus hudsonicus* declined after Unit 8-9. *Tamias* spp. increased in Unit 6 (1,389 cal YBP, 95 % CI = 1,919 – 879 cal YBP), *Zapus princeps* returned in Units 2 (502 cal YBP, 95 % CI = 603 – 367 cal YBP) and both *Z. princeps* and *Ochotona princeps* were found in Unit 1 (257 cal YBP, 95 % CI = 446 – -52 cal YBP).

Overall, the community experienced a shift between Units 12-13 and 10, and again between Units 9A and 8-9, reflected in the change in proportion of closed versus open habitat taxa through time (Figure 5). Closed habiat taxa were more abundant than open habitat taxa in Units 16 (3,553 cal YBP, 95 % CI = 5,288 – 2,818 cal YBP) and 15 (2,950 cal YBP, 95 % CI = 3,726 – 2,773 cal YBP) where there was no overlap in confidence intervals, and likely in Unit 14 (2,784 cal YBP, 95 % CI = 3,099 – 2,698 cal YBP). In Units 12-13 through 9A (2,594 – 1,627 cal YBP, 9 5% CI = 2,721 – 1,380 cal YBP), open and closed habitat taxa were more or less equally abundant, but the proportion of closed habitat taxa did not decline from before. In Units 8-9 (1,580 cal YBP, 95 % CI = 2,256 – 1,237 cal YBP) and 6 (1,389 cal YBP, 95 % CI = 1,919 – 879 cal YBP), however, open and closed habitat taxa were still equally abundant, but closed habitat taxa declined sharply. Beginning in Unit 4 (927 cal YBP, 95 % CI = 1,245 – 692 cal YBP), open habitat taxa were more abundant than closed habitat taxa, and the proportion of closed habitat taxa remained lower than in the earlier half of the record.

Changes in nocturnality and diurnality of small mammals. Although the relative abundance of nocturnal and diurnal species fluctuated through time (Figure 5), nocturnal taxa were more abundant across the record, and significantly so for most units. The abundance of taxa with no diel preference declined from Unit 16 through Unit 10, then remained stable, and low, through time. There was very slight overlap in the Goodman's 95 % confidence intervals for relative abundances of nocturnal and diurnal taxa in Units 12-13 (2,594 cal YBP, 95 % CI = 2,721 – 2,455 cal YBP), 10 (2,417 cal YBP, 95 % CI =2,585 – 2,139 cal YBP), 9A (1,627 cal YBP, 95 % CI = 2,308 – 1,380 cal YBP), and 4 (927 cal YBP, 95 % CI = 1,245 – 692 cal YBP), but nocturnal taxa were more common overall. The only exception was Unit 12-13 where relative abundances of nocturnal and diurnal taxa were very similar (53 % and 47 % respectively) and there was considerable overlap in confidence intervals.

Influence of climate on mammal community composition. Our model shows that, early in the record (~3,400 – 3,000 cal YBP), annual temperatures increased, mainly driven by increases in winter temperature, while annual precipitation declined (Supplemental 5). From ~3,000 – 2,000 cal YBP, winter (and annual) temperature declined again while precipitation declined slightly. From ~2,000 – 1,000 cal YBP, winter (and annual) temperature increased sharply then declined again, and precipitation was lower than previously. After ~1000 cal YBP, summer temperature increased, associated with the onset of the Medieval Climate Anomaly (MCA), but by ~500 cal YBP winter temperature decreased, associated with the Little Ice Age. The net effect was an

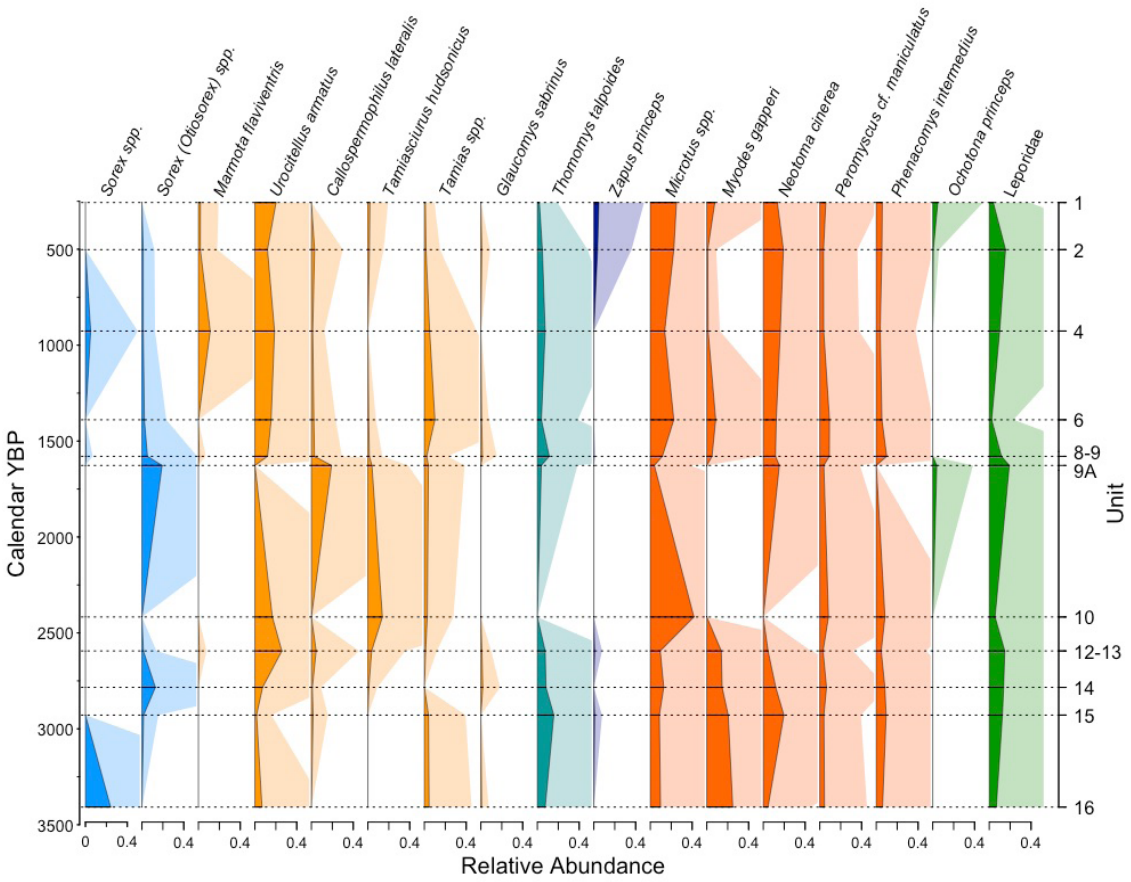

Figure 4. Waterfall Locality changes in small mammal relative abundance over time. Darker curves show relative abundance, lighter curves are an exaggeration of 8x to emphasize rare taxa. Taxa are organized by order/family: blue = Soricidae; yellow = Sciuridae; teal = Geomyidae; purple = Zapodidae; orange = Cricetidae; green = Lagomorpha.

increase in temperature seasonality over the last 1000 years, paired with a continued decline in precipitation and increasing summer temperature.

In our analysis of the relationship between climate variables and measures of both diversity (richness and evenness) and community composition (proportion of open- and closed-habitat taxa), which combined age model resampling and permutation tests, we found that precipitation was an important factor. Although most metrics were significantly more correlated with climate than random, the strength of the correlation, reflected in the Pearson's correlation coefficient, was very low (< |0.1|) for most relationships, indicating no effective relationship (Table 4; Supplemental 5). However, annual precipitation was slightly more strongly negatively correlated with SQS (mean correlation coefficient = -0.22) and the proportion of open habitat taxa (correlation coefficient = -0.32). We saw a stronger signal with summer precipitation, which was negatively correlated with the proportion of open habitat taxa (mean correlation coefficient = -0.42) and positively correlated with the proportion of closed habitat taxa (mean correlation coefficient = -0.37). Overall, this suggests that higher summer precipitation contributes to a higher proportion of closed habitat taxa, at the expense of taxa which favor open habitats.

The diastema length of fossil *Thomomys talpoides* from Waterfall Locality ranged from 6 to 9 mm, with a mean of 6.75 mm (Supplemental 6). Sample size was too small to establish a trend through time or with climate, but diastema length was larger in Units 14 (2,784 cal YBP, 95 % CI = 3,036 – 2,705 cal YBP) and 8-9 (1,580 cal YBP, 95 % CI = 2,256 – 1,237 cal YBP), and smaller in Units 12-13 (2,594 cal YBP, 95 % CI = 2,721 – 2,455 cal YBP) and 4 (927 cal YBP, 95 % CI = 1,245 – 692 cal YBP), both of which only had N = 1. The diastema length for fossil *T. talpoides* from Waterfall Locality was larger on average than both fossil and modern populations at nearby Lamar Cave (1,835 m), which is lower in elevation than Waterfall Locality (Hadly 1997). Waterfall Locality *T. talpoides* had similar diastema sizes to modern

Table 4. Mean Pearson's product moment correlation coefficients for correlations of climate and diversity metrics. For comparisons with no value (—), the distribution of correlation coefficients was not significantly different from random, as determined by a permutation test. SQS = shareholder quorum subsampling species richness; PIE = Probability of intraspecific encounter species richness; open = relative abundance of open habitat-associated taxa; closed = relative abundance of closed habitat-associated taxa.

Climate Variable	SQS	PIE	Open	Closed
Mean annual temperature	-0.10	0.10	-0.10	-0.02
Minimum winter temperature	—	0.02	-0.11	0.04
Maximum summer temperature	0.11	—	0.03	-0.07
Temperature seasonality	0.13	-0.02	0.10	-0.12
Annual precipitation	-0.22	0.10	-0.32	0.16
Mean summer precipitation	-0.17	-0.10	-0.42	0.37
Precipitation seasonality	0.08	-0.05	0.04	-0.05

specimens from nearby populations at higher elevations such as Canyon (2,590 m) and Cooke City (2,560 m), which averaged from 6.5 to 7.0 mm (Hadly 1997).

Influence of wildfire on mammal community composition. Estimated number of fire events (*i. e*., charcoal pieces) averaged zero to one per century from 3,300 – 2,200 YBP, then increased to an average of one to two per century from 2,200 – 1,400 cal YBP (Figure 5). From 1,400 – 1,200 YBP, the average number of charcoal pieces per century was zero but increased again to an average of one to two per century from 1,200 – 400 cal YBP, before returning to an average of zero per century from ~400 cal YBP to present.

Discussion

Large fires that alter habitat cover in the Yellowstone ecosystem have burned periodically since the last glacial period (Millspaugh *et al.* 2000; Huerta *et al.* 2009; Higuera *et al.* 2010; Power *et al.* 2011). Reconstruction of the mammal community from Waterfall Locality offers a ~3,400-year record of mammal diversity in lower montane ecosystems of the northern Rocky Mountains, linked to vegetation type, climate, and fire regime change over time. Although past research has explored the relationship between these factors in the northern Rocky Mountains over paleoecological time scales, vertebrate fossil localities are rare in this system and so there has been less attention to the ways in which the biota has responded to these long-term changes. Understanding linked abiotic and biotic dynamics is important for anticipating how these taxa will respond to future climate and fire regime change.

While vegetation type is a major determinant of animal diversity in general, small mammals are also very responsive to fire (e. g., Culhane *et al.* 2022). Thus, comparisons of small mammal communities across sites can be a robust indicator of changes in vegetation structure and composition (*i. e*., Grant and Birney 1979; Culhane *et al.* 2022). Mammals found in Waterfall Locality deposits are typical of the Yellowstone region today and include taxa associated with grassland, sagebrush, forest, and riparian habitats (Supplemental 4, Hadly 1999). *Microtus* spp. (voles), *Urocitellus armatus* (Uinta ground squirrel), and leporids (rabbits and hares) persisted throughout the deposit. *Urocitellus armatus* is characteristic of sagebrush grassland with sparse cover (Streubel 1995; Barnosky 1994; Hadly 1996, 1999; Craighead 2000) and leporids are often indicators of dry shrublands and ecotones, except *Lepus americanus* (snowshoe hare) which is found in closed forest (Streubel 1995; Hadly 1996). Most of the *Microtus* specimens from Waterfall Locality were not identified to species and thus we were not able to track turnover in *Microtus* species through time, but we know that all four species found in Yellowstone today were also present in Waterfall Locality: *M. pennsylvanicus* (meadow vole) and *M. richardsonii* (water vole) were morphologically identified and found sporadically, while *M. longicaudis* (long-tailed vole) and *M. montanus* (montane vole) were confirmed using aDNA in a study by Spaeth *et al.* (2009). Of the 15 samples in this genetic dataset, 12 belonged to *M. longicaudis* while only 3 were *M. montanus*. While all four Yellowstone *Microtus* species are common in wet meadows and grasslands (Viteri *et al.* 2021; Streuble 1995), the high numbers of *M. longicaudis* relative to *M. montanus* are consistent with a forested environment (Anich and Hadly 2013). These taxa provide evidence that grassland and shrubland environments were consistently present in the vicinity of Waterfall Locality over the last ~3,400 years, as they are today (Figure 1C and D). Closed habitat/forest-associated species are also found throughout the deposit, for example *Tamiasciurus hudsonicus* (red squirrel), *Glaucomys sabrinus* (flying squirrel), and *Myodes gapperi* (Southern red-backed vole).

Based on the mammal community, it is clear that over the last ~3,400 years there were changes in the relative proportion of habitats within the sampling radius of Waterfall Locality. We found an overall decline in the proportion of mammal taxa associated with closed habitats (*i. e*., forests), coincident with an increase in taxa associated with open habitats (grasslands and shrublands) (Figure 5). These changes in the small mammal community were likely mediated by vegetation changes that led to a greater proportion of open environments in the vicinity of the site. Forested habitat was most widespread around Waterfall Locality before ~ 2,800 cal YBP, but open environments began to expand after ~2,600 cal YBP and eventually became dominant after ~1,500 cal YBP. We also found that higher summer precipitation was significantly positively correlated with the proportion of closed habitat taxa (and negatively correlated with the proportion of open habitat taxa). Vegetation reconstructions using pollen from sediment cores from nearby Slough Creek Pond, ~23 km to the west, show that there was a regional shift from forests dominated by lodgepole pine, limber pine, and juniper, to Douglas-fir parkland (more open environments with low tree density and non-continuous tree cover) beginning as early as 7,000 cal YBP, but especially after 3,000 cal YBP (Millspaugh 1997, Whitlock and Bartlein 1993). As aridity increased from 4,000 – 2,000 YBP, sagebrush steppe and *Pseudotsuga* (Douglas-fir) and *Pinus contorta* (lodgepole pine) parkland expanded, and grasses increased (Whitlock and Bartlein 1993, Huerta *et al.* 2009, Whitlock *et al.* 2012). At Crevice Lake, further northwest but lower in elevation (576 m below Waterfall Locality), vegetation trends were similar to those at Slough Creek Pond: from ~8,200-2,800 cal YBP, Douglas-fir parkland increased, *Pinus* and *Juniperus* declined, and summers became drier over this period; from 6,000 to 2,000 cal YBP in particular, xerophytic forest expanded (Whitlock *et al.* 2012). A similar transition seems to have taken place at Waterfall Locality around 2,800 cal YBP, when we saw an initial increase in open habitat taxa in Unit 14. At Lamar Cave, ~28 km west of Waterfall Locality and 400 m lower in elevation, Hadly (1996) noted a warm climatic period from 2,850 to 2,050 cal YBP which likely contributed to this shift.

Individual taxa also corroborate our conclusion that open habitat expanded around Waterfall Locality as the climate became drier. Up until ~2,600 cal YBP, we found higher abundances of taxa associated with closed forests (*e. g.*, *Glaucomys sabrinus*, *Lepus americanus,* and *Myodes gapperi*). *Thomomys talpoides* (northern pocket gopher) and *Zapus princeps* (western jumping mouse), which rely on grassy habitats (Streubel 1995; Barnosky 1994), were also found through this time, possibly suggesting the presence of montane meadows in the vicinity. The community *ca.* 2,600 cal YBP (Unit 12-13) had particularly diverse representation of mesic and closed forest-associated taxa, including *Mustela frenata* (long-tailed weasel), *Lepus americanus*, *Thomomys talpoides*, and *Zapus princeps* (Hadly 1996, 1997). Between ~2,400 and ~1,600 cal YBP, these mesic/forest species declined, and we saw increases in sciurids, like *Urocitellus armatus,* found in sagebrush and grassy habitats (Hadly 1996; Wilson and Ruff 1999); *Callospermophilus lateralis* (golden-mantled ground squirrel), which prefer mountain meadows, rocky habitats, forest edge and open woodland environments; and *Tamiasciurus hudsonicus*, which rely on spruce, fir, and pine forests (Wilson and Ruff 1999). An increase in xeric species is corroborated by the diatom ecology of Crevice Lake, ~40 km to the west, where summers from 2100 to 800 cal YBP likely began earlier and lasted longer (Whitlock *et al.* 2008). In sum, increases in these taxa suggest drying and opening habitats, a likely increase in sagebrush habitats, with persistence of some forests.

Forests may have recovered somewhat after ~1,600 cal YBP (beginning in Unit 8-9): *Myodes gapperi* reappeared and *Phenacomys intermedius* (western heather vole), also usually associated with forest environments, increased. Around 1,580 cal YBP (Unit 8-9) we also saw an increase in the more mesic indicator species *Thomomys talpoides* and brief return of *Lepus americanus* and *Zapus princeps*. However, aridification and expansion of open environments generally continued after this. *Marmota flaviventris* (yellow-bellied marmot) increased in abundance around 930 cal YBP (Unit 4). Although the primary habitat requirement of *M. flaviventris* is rocky outcrops, they are also associated with drier open habitats like sagebrush grassland and Douglas-fir parkland rather than closed forest. At Lamar Cave, the small mammal community reflects similar changes: beginning around 1,200 cal YBP and throughout the Medieval Climate Anomaly (MCA; ~1,000 – 650 cal YBP), xeric indicator taxa were more abundant than mesic indicator taxa, although mesic taxa recovered somewhat during the Little Ice Age (LIA; ~700 – 100 cal YBP). For example, the mesic taxa *Microtus montanus* and *Thomomys talpoides* both declined at Lamar Cave during the MCA then increased again during the LIA (Hadly 1996). Regional drought was severe enough during the transition from the MCA to the LIA (specifically, 717-588 cal YBP), that the Old Faithful Geyser ceased to erupt due to insufficient water (Hurwitz *et al.* 2020). This overall pattern matches the significantly higher proportion of open habitat taxa in Waterfall Locality around 930 cal YBP (Unit 4), corresponding to the MCA, as well as the slight decline in open habitat taxa around 500 cal YBP (Unit 2) and 260 cal YBP (Unit 1), corresponding to the cooler and wetter LIA (Figure 5). Indeed, moraines in the Soda Butte Creek drainage suggest that there may have been a local glacial advance during the LIA (Meyer 1995b). Several mesic/closed habitat taxa returned in Units 1 and 2, including *Mustela frenata*, *Lepus americanus*, and *Ochotona princeps* (American pika), while *Marmota flaviventris* declined (Table 2, Figure 3). However, *Zapus princeps* increased markedly after ~ 500 cal YBP, indicating that grasslands were still expanding, possibly corresponding to a slightly more mesic time during the LIA (Figure 5). Around 250 cal YBP, *Myodes gapperi* increased, suggesting increase in forest cover, consistent with emergence of larger, older trees after the LIA (Figure 3).

One incongruous pattern is that *Thomomys talpoides* declined in abundance from the MCA around 930 cal YBP (Unit 4) through the LIA around 500 cal YBP (Unit 2) and 260 cal YBP (Unit 1), which is counter to the trends seen at Lamar Cave, where *T. talpoides* was more abundant during the LIA than during the MCA (Hadly 1997). Although *T. talpoides* in the Yellowstone area are known to increase in abundance during more mesic times likely because of expansion of highly productive forbs (Hadly 1997), they are not forest species, instead preferring ecotones without dense tree roots. Thus, the decline in *T. talpoides* may indicate the maturation of the surrounding trees in the forest. The higher abundance of *T. talpoides* ~1,580 cal YBP (Unit 8-9) also corresponds to the largest diastema lengths, although our sample sizes were low (Supplemental 6). Diastema length (a proxy for body size) in the Yellowstone region generally correlates with elevation (Hadly 1997), and previous research has shown that the direct cause is nutritional quality (Smith and Patton 1988). The larger diastema at Waterfall Locality around 1,580 cal YBP (Unit 8-9), coupled with higher abundance, could correspond to the opening of forest and greater availability of ecotones and open habitat favorable for pocket gophers. Diastema length was also larger *ca*. 2,780 cal YBP (Unit 14), a period we interpret as having more closed forest. One possibility is that during this timeframe, the *T. talpoides* were coming from nearby alpine grassland (Figure 1 B-D). The topography around Waterfall Locality is very steep, so high elevation habitats are much more proximate than at Lamar Cave, for example.

Changes in taphonomy can influence the relative abundance of taxa in the deposit as compared to taxa on the landscape, obscuring interpretations of the true mammal community change (Hadly 1999). Possible taphonomic biases at Waterfall Locality include the following: 1) changes in the sampling radius through time such that more or fewer habitats are sampled, 2) changes in prey selection by predators over time, and 3) changes in predators through time. Porder *et al.* (2003) reconstructed the sampling radius of Lamar Cave and Waterfall Locality by comparing the Strontium (Sr) isotopes of bones found in these two fossil

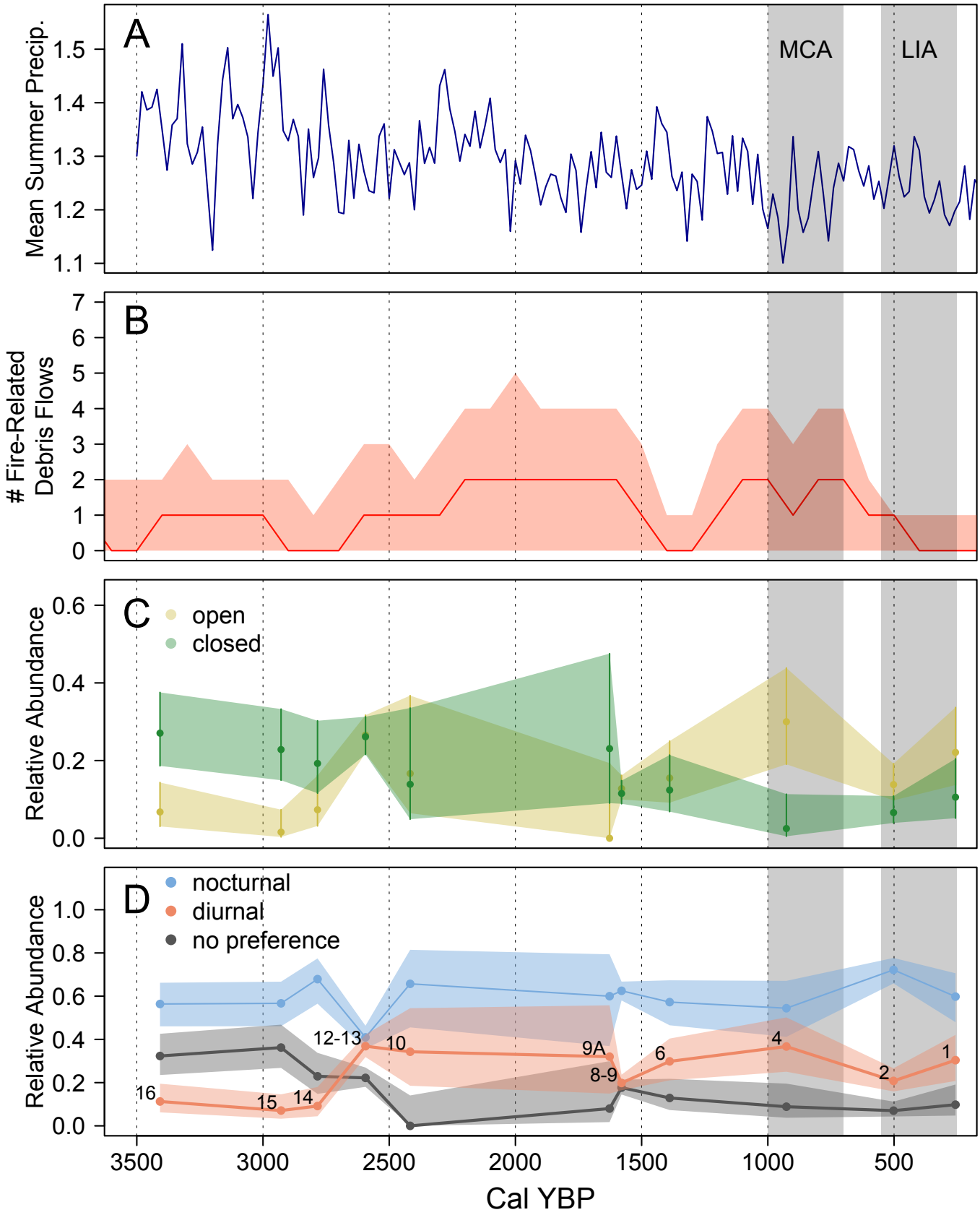

Figure 5. Precipitation, fire, and small mammal abundance change. A) Mean summer precipitation averaged over 30-year intervals at 20-year steps, for latitude 42.5 to 45.0° N, longitude 112.5 to 110.0° W, from PaleoView (Fordham *et al.* 2017). Vertical gray shaded areas show the approximate timing of the Medival Climate Anomaly (MCA) and Little Ice Age (LIA) based on Mann *et al.* (2009). B) Estimated fire-related debris flows based on average number of charcoal samples per 100 years (red line); shaded area shows the 95 % confidence interval. Relative abundance of C) open-habitat and closed-habitat taxa and D) nocturnal and diurnal taxa, and taxa with no diel preference. Solid lines and points show relative abundance; shaded polygons are Goodman's Simultaneous Confidence Intervals. Numbers shown in D) correspond to excavation unit numbers that apply to both C and D.

sites to Sr isotopes in vegetation around the site. The study showed that the collection radius of Lamar Cave, which has a geologic substrate that permitted a more fine-grained assessment, was 8 km or less. The collection radius around Waterfall Locality, with a more homogeneous bedrock in the vicinity, is 17.5 km or less. Neither site demonstrated a change in radius through time (Porder *et al.* 2003). With respect to changes in prey selection, Viteri *et al.* (2021) demonstrated that variation in the small mammal diet of raptor species in the Yellowstone region was driven by site rather than by raptor identity, that is, raptor species sample available diversity on the landscape rather than preferentially selecting certain prey species. We analyzed the composition of mammals in Waterfall Locality to determine if there had been changes in the proportion of nocturnal versus diurnal taxa, which would indicate a shift in predator identity, from nocturnal predators like owls to diurnal predators like avian raptors. We found that nocturnal taxa were consistently more common through time and the proportion was generally stable (Figure 5). However, the proportion of diurnal taxa was low from ~3,400 through ~2,780 cal YBP (Units 16 through 14), then increased and remained higher from ~2,590 through ~260 cal YBP (Units 12-13 through 1). This shift was driven by a decline in taxa with no diel preference. While the stability of nocturnal taxa suggests that there was no change in taphonomic biases influencing species abundance in Waterfall Locality, the increase in diurnal taxa may indicate an increase in avian raptors excluding owls. This is consistent with our interpretation of a closed forest habitat from ~3,400 (Unit 16) through ~2,780 (Unit 14) or ~2,590 cal YBP (Unit 12-13), because diurnal avian raptors are not typical in closed forest communities. These predators would likely have increased as the forest opened after ~2,590 cal YBP (Unit 12-13).

The modeled climate record suggests that, over the last ~3,400 years, climate changes were nonlinear and the various components of climate (temperature, precipitation, seasonality, etc.) changed in different ways at different times (Supplemental 5). However, while our analyses show that community composition was influenced by changes in precipitation, other climate factors did not have an evident impact on species richness (SQS) or evenness (PIE) based on our Pearson's correlations and permutation tests. One possible explanation for the absence of an effect of climate on diversity is time-averaging and/or time-transgression within each unit. Duplicate and triplicate radiocarbon dates on Units 2, 6, 9, 10, 13, and 14 suggest that the excavation units encompass from ~300 to ~1100 years in time-averaging or time-transgression, and this may mask fine-scale signals of climate change in the faunal assemblage. However, we did detect an effect of precipitation on open versus closed habitat taxa, suggesting that it would have been possible to find climatic effects on richness and evenness if they had been present. Given the relatively coarse spatial scale (2.5° latitude x 2.5° longitude) of the PaleoView climate data used here, spatial averaging may also dampen our ability to detect relationships between climate and diversity, especially in a topographically complex region like our study area.

Our examination of the Meyer *et al.* (1995a) charcoal data, in which single charcoal pieces from individual fire-related debris flows were dated, shows increased numbers of fire-related debris flows from ~2,200 – 700 cal YBP, except from ~ 1,500 – 1,200 cal YBP when our estimate declined sharply (Figure 5). The increase in charcoal that we reconstructed coincides with the possible increase in open habitat taxa between ~ 2,420 and 1,630 cal YBP (Units 10 and 9A; Figure 5), suggesting that wildfire may have cleared the existing forests, and then either forest regeneration was inhibited/reduced by the more arid conditions, or forest was replaced with more open treed environments, like Douglas-fir parkland. Although there is some overlap in the timing of peak fire between Meyer *et al.* (1995a) and our reanalysis of the data—Meyer *et al.* (1995a) identified times of elevated fire-related debris flows from 2,300 – 2,050 YBP and 900 – 750 YBP in the Soda Butte Creek and Slough Creek drainages—the differences are likely due to the difference in method: Meyer *et al.* (1995a) used summed probability distributions to estimate fire frequency, whereas we estimated the number of fires from the number of debris flows (each dated with a single charcoal piece) and incorporated age uncertainly by resampling the calibrated age distributions.

Brown *et al.* (2020) found that fires in the Beartooth Mountains northeast of Waterfall Locality were more frequent but less severe in low elevation Douglas-fir and lodgepole pine-dominated vegetation than they were in higher elevation whitebark pine forests, so the sustained ~800-year period of elevated fire we found from ~2,300-1,500 cal YBP may have been reinforced by changing vegetation (Figure 5). There was a sharp decline in the mean estimate for taxa associated with closed/forested habitats around 1,580 cal YBP (Unit 8-9), coincident with the sharp decline in fire frequency. One plausible scenario is that wildfire burned a large proportion of the forest around Waterfall Locality immediately prior to this time, reducing the number of forest-associated mammals and also reducing fuels which could have supplied subsequent fires. Following this, fire frequency increased again from ~1,100 to 700 cal YBP, but open habitat taxa remained more common than closed habitat taxa, so fires may have been taking place in the open habitats like grasslands and sagebrush steppe. Additionally, the tree cover in these forests takes centuries to mature. These patterns roughly match estimated fuel biomass at Crevice Lake, where there were more fuels from ~4,500 – 1,600 cal YBP, and less from 1,600 – 300 cal YBP (Whitlock *et al.* 2012). Additional paleo-fire records from near Waterfall Locality will refine these interpretations. Sedimentary charcoal from Foster Lake, near the confluence of Soda Butte Creek and the Lamar River (Firmage 2019), is one such record, but uncertainty in the age model for Foster Lake makes comparison difficult.

Modern small mammal abundance patterns support the idea that elevated wildfire may have caused faunal turnover at Waterfall Locality. Wood (1981) surveyed small mammal communities in Yellowstone following wildfire and found that *Tamias amoenus* was more common in recently burned sites than in unburned control sites. At Waterfall Locality, *Tamias* spp. were more common from ~1,390 to ~930 (Units 6 and 4), consistent with elevated wildfire in the area from ~1,200 – 700 YBP (Figure 4 and 5). In a meta-analysis of small mammal abundance following wildfire, Zwolak (2009) found decreases in *Myodes gapperi*. At Waterfall Locality, *Myodes gapperi*, a closed/forest-associated species, was found in higher abundance from ~3,400 – 2,590 YBP (Units 16 through 12-13) than after ~2,420 (Unit 10 and above) when there were more fires (Figure 4 and 5). Numerous authors have found increases in *Peromyscus maniculatus*, a ubiquitous generalist species, following wildfires in North American coniferous forests (*e. g.*, Wood 1981; Bunnell 1995; Converse *et al.* 2006; Zwolak 2009), but *P. maniculatus* varied little through time at Waterfall Locality. Bunnell (1995) showed that mammals were more impacted by fire size and hectares burned per year than by fire return interval, so reconstruction of fire events from charcoal—which does not reflect fire size—may provide only a partial picture. Nevertheless, our data suggest that changes in climate coupled with changes in fire frequency have long-term impacts on small mammal communities by altering habitat availability.

Mammalian species found in Waterfall Locality are common in Yellowstone today, and include taxa from grassland, forest, and riparian habitats. The persistence of these taxa throughout the Waterfall midden suggests that these environments were consistently present in the vicinity of Waterfall Locality over the last ~3,400 years but changed in their relative proportion through time. Our analyses of mammal fossil material from Waterfall Locality over the last ~3,400 years show that changes in the small mammal community—in terms of the relative abundance of individual taxa as well as taxa associated with open versus closed habitats—tracked changing summer precipitation and possibly changes in fire frequency. The small mammal community indicated a higher proportion of forested and more mesic habitats from ~3,400 to ~2,800 cal YBP. Increasing proportions of small mammals preferring open habitats, and species particularly associated with sagebrush, grasslands, and open habitats were seen from ~2,600 to ~900 cal YBP. From ~500 to ~250 cal YBP, within excavation units deposited during the Little Ice Age, we saw the return or increase of some mesic and forest-habitat mammals. Changes in the fire regime may have caused a shift toward more open-habitat taxa; further research would improve our ability to match the paleofire and fossil mammal data. Waterfall Locality represents a ~3,400-year record of mammal diversity in lower montane forests of the northern Rockies and indicates how the mammal community will adjust to changing climate and fire regimes mediated by habitat change.

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Riparian woodrat and black rat competition: investigating the role of an exotic species in the decline of a native endangered species

Brian L. Cypher[1]*, Patrick A. Kelly[1], Karen L. Sproull[1], Daniel F. Williams[1], and Scott E. Phillips[1]

[1] Endangered Species Recovery Program, California State University-Stanislaus, One University Circle, 95382, Turlock. California, USA. Email: bcypher@esrp.csustan.edu (BLC); pkelly@csustan.edu (PAK); biogirl999@gmail.com (KLS); danw1942@gmail.com (DFW); sphillips@esrp.csustan.edu (SEP).

* Corresponding author: https://orcid.org/0000-0002-7349-545X.

Competition from non-native species constitutes a significant threat to numerous native species worldwide. Endangered riparian woodrats (*Neotoma fuscipes riparia*) may be restricted to a single population occupying approximately 100 ha at Caswell Memorial State Park (CMSP) in central California. This population is vulnerable to a number of threats including from non-native black rats (*Rattus rattus*) that co-occur at CMSP. Black rats potentially engage in both interference and exploitative competition with woodrats. From September 2001 to September 2004, we investigated interactions between riparian woodrats and black rats to determine whether competitive interactions were reducing woodrat abundance or reproductive success. Two sites with riparian woodrats were identified at CMSP. Between January 2003 and September 2004, 179 black rats were removed from one site. Abundance of both species and woodrat reproductive success were assessed through live-trapping and radio-telemetry. Mean litter size, mean number of young for litters with emerged young, and mean number of young per female were all higher on the black rat removal site compared to the control site. Also, mean number of young for litters with emerged young increased on the removal site from 2003 to 2004 and decreased on the control site between years. Woodrat abundance trends were more equivocal and actually were higher on the control site during most of the period of black rat removal. The results of this investigation suggest that black rats may indeed suppress reproductive success of riparian woodrats, and that black rat removal could benefit woodrats. Black rats have been implicated in the declines and even extinction of other native rodents, including other woodrat species. Thus, black rats may constitute a significant threat to riparian woodrats, particularly in concert with other threats such as flooding, wildfires, and continued habitat loss and degradation. Therefore, we recommend that black rats be removed quarterly from highly suitable woodrat habitat in the CMSP. We also recommend that surveys be conducted to identify additional riparian woodrat populations and that black rat removals be conducted in those populations as well.

La competencia entre especies exoticas constituye una amenaza significativa para numerosas especies nativas en todo el mundo. Las ratas montera ribereñas (Neotoma fuscipes riparia) considerada en peligro de extinción pueden estar restringidas a una sola población que ocupa aproximadamente 100 ha en el Parque Estatal Caswell Memorial (CMSP) en el centro de California. Esta población es vulnerable a una serie de amenazas, incluidas las de ratas negras introducidas (Rattus rattus) que coexisten en CMSP. Las ratas negras potencialmente participan tanto en interferencia como en competencia de explotación con las ratas del bosque. Desde septiembre de 2001 hasta septiembre de 2004, investigamos las interacciones entre rata montera ribereñas y ratas negras para determinar si las interacciones competitivas han reducido la abundancia de ratas monteras o el éxito reproductivo. Se identificaron dos sitios con ratas monteras ribereñas en CMSP. Entre enero de 2003 y septiembre de 2004 y se retiraron 179 ratas negras de un sitio. Se evaluó la abundancia de ambas especies y el éxito reproductivo de las ratas monteras mediante trampas vivas y radiotelemetría. l tamaño promedio de camada, el promedio de crías emergidas por camadasy el promedio de crías por hembra fueron más altos en los sitios con remoción ratas negras en comparación con el sitio de control. Además, el promedio de crías en las camadas con crías emergidas aumentó en el sitio de eliminación de 2003 a 2004 y disminuyó en el sitio de control en esos años. Las tendencias de abundancia de las ratas monteras fueron más equívocas y de hecho fueron mayores el sitio de control durante la mayor parte del período de eliminación de las ratas negras. Los resultados de esta investigación sugieren que las ratas negras pueden efectivamente suprimir el éxito reproductivo de las ratas monteras ribereñas, y que la eliminación de las ratas negras podría beneficiar a las ratas monteras. Las ratas negras han estado implicadas en la disminución e incluso la extinción de otros roedores nativos, incluidas otras especies de ratas monteras. Por lo tanto, las ratas negras pueden constituir una amenaza significativa para las ratas monteras ribereñas, particularmente en conjunto con otras amenazas como inundaciones, incendios forestales, la pérdida y degradación continua del hábitat. Por tanto, recomendamos que las ratas negras sean removidas trimestralmente de hábitats altamente adecuados a las ratas monteras en el CMSP. También recomendamos que se realicen estudios para identificar poblaciones adicionales de ratas monteras ribereñas y que se realicen eliminaciones de ratas negras en esas poblaciones si se justifica.

Keywords: Abundance; California; competition; endangered species; live-trapping; *Neotoma fuscipes riparia*; *Rattus rattus*; reproduction.

Introduction

The range of the dusky-footed woodrat (*Neotoma fuscipes*) extends from northwestern Oregon to south-central California (California Wildlife Habitat Relationships 2024). One subspecies, the riparian woodrat (*N. f. riparia*), is restricted to riparian habitat in the northern San Joaquin Valley of California (Figure 1a). Due to profound loss of riparian habitat, the current range of the riparian woodrat is primarily confined to a single site, Caswell Memorial State Park (CMSP; Figure 2), comprising approximately 100 ha (250 ac) along the Stanislaus River in Stanislaus County (Williams 1986). Since 2003, riparian woodrats have also been documented periodically on the San Joaquin River National Wildlife Refuge and one or more other small populations also persist along the Stanislaus and San Joaquin Rivers (USFWS 2020). Overall, the geographic range is poorly understood. Historically, this subspecies may have occurred from the San Francisco East Bay region to Fresno County in the central San Joaquin Valley (Hooper 1938). However, recent analyses suggest that the phylogeographic relationships of woodrat populations in central California is more complicated than previously thought (Matocq 2002a, 2002b). Matocq *et al.* (2012) show that the CMSP population exhibits a hybrid ancestry with *N. macrotis* populations to the east in the Sierra Nevada. The very restricted distribution of *N. f. riparia*, in conjunction with various threats, resulted in the subspecies being listed as Federally Endangered in 2000 (USFWS 2000). It also is considered to be a Species of Special Concern by the State of California (Williams 1986).

Riparian woodrats occur in riparian forest habitats with an overstory dominated by valley oaks (*Quercus lobata*) and a dense shrub understory consisting of willow (*Salix* spp.), wild rose (*Rosa californica*), blackberry (*Rubus* spp.), wild grape (*Vitis californica*), and coyote bush (*Baccharis* spp.). Within these habitats, woodrats typically construct terrestrial stick nest houses measuring 0.6 to 0.9 m high and 1.2 to 1.8 m in diameter (Lindsdale and Tevis 1951; Williams 1993) but may also construct nests in the tree canopy, tree cavities, and downed logs. Each house is typically occupied by a single adult. Females produce 1 to 5 litters per year, comprising 3 to 4 young each. Reproduction can occur in all months with pregnancy rates being highest in February (Williams *et al.* 1992). Riparian woodrats are arboreal, primarily nocturnal, and generalist herbivores with a diet consisting of leaves, fruits, shoots, flowers, nuts, and fungi (Williams *et al.* 1992). Predators of riparian woodrats may include coyotes (*Canis latrans*), gray foxes (*Urocyon cinereoargenteus*), long-tailed weasels (*Mustela frenata*), mink (*Neovison vison*), raccoons (*Procyon lotor*), feral cats (*Felis catus*), bobcats (*Lynx rufus*), owls (Strigidae) and other raptors (Williams 1988).

Loss and degradation of riparian forest habitat is the primary cause of the decline of riparian woodrat populations and the primary threat to the continued existence of the subspecies. Over 95% of this habitat type in the Central Valley has been destroyed (Katibah 1984; Kelly *et al.* 2005) and by about 1980, only ~269 ha of mature riparian forest remained in the San Joaquin Valley (Williams and Kilburn 1984). Remaining old growth riparian habitat tends to be fragmented and degraded. However, since 2001 there has been a concerted effort to restore riparian habitat in some areas of the northern San Joaquin Valley (*e.g.*, at the San Joaquin River National Wildlife Refuge). Threats to remaining habitat include flooding and wildfire (Close and Williams 1998), and CMSP has experienced multiple floods and wildfires in recent years, including a major fire in July 2022. Additional threats to the remaining riparian woodrat population include elevated predation pressure (*e.g.*, from feral carnivores), disease, potential inbreeding depression, and demographic stochasticity (USFWS 1998). Williams (1993) estimated a peak population of 437 riparian woodrats.

An additional potential threat to riparian woodrats is competition from non-native black rats (USFWS 2000; Figure 1b). Black rats (*Rattus rattus*) are extremely adaptable and can readily colonize anthropogenically-altered habitats. Black rats are opportunistic omnivores and often exhibit high fecundity (Invasive Species Specialist Group 2008). Being highly arboreal, they can go anywhere that woodrats go, probably including the nest chamber of woodrat houses while the occupant is away foraging in the canopy. Consequently, black rats could potentially engage in both interference and exploitation competition with riparian woodrats. Interference competition could be in the form of spatial or temporal avoidance by woodrats, perhaps via chemical communication (Brown *et al.* 1996), or even direct mortality to woodrats, particularly juveniles and nestlings. Exploitation competition could be in the form of overlapping food habits or usurping of woodrat houses and food caches by black rats.

Black rats are often documented in riparian habitat throughout the Central Valley and they are abundant at CMSP. In 1993, trapping efforts at CMSP resulted in the capture of 57 riparian woodrats and 52 black rats (Williams 1993). In 2000, trapping the same areas at CMSP as in 1993, and with approximately similar levels of effort, resulted in the capture of 12 riparian woodrats and 109 black rats (CSU Stanislaus, Endangered Species Recovery Program, unpublished data). Thus, black rats appeared to have substantially increased over a short time period at CMSP.

A thriving black rat population at CMSP is a concern because, although the effects of black rats on riparian woodrats are unknown, black rats are known to significantly impact insular ecosystems worldwide (Stapp 2002; Thibault *et al.* 2002; Major *et al.* 2006; Caut *et al.* 2008). Also, in Florida, an inverse relationship was found between the abundance of black rats and endangered Key Largo woodrats (*Neotoma floridana smalli*), and no female woodrats were captured in areas occupied by black rats (Sasso and Gaines 2002). In the San Francisco Bay Area, there are concerns that non-native rats may be expanding and replacing woodrats in parts of the East Bay Regional Parks District (J. Patton, University of California-Berkeley, pers. comm.).

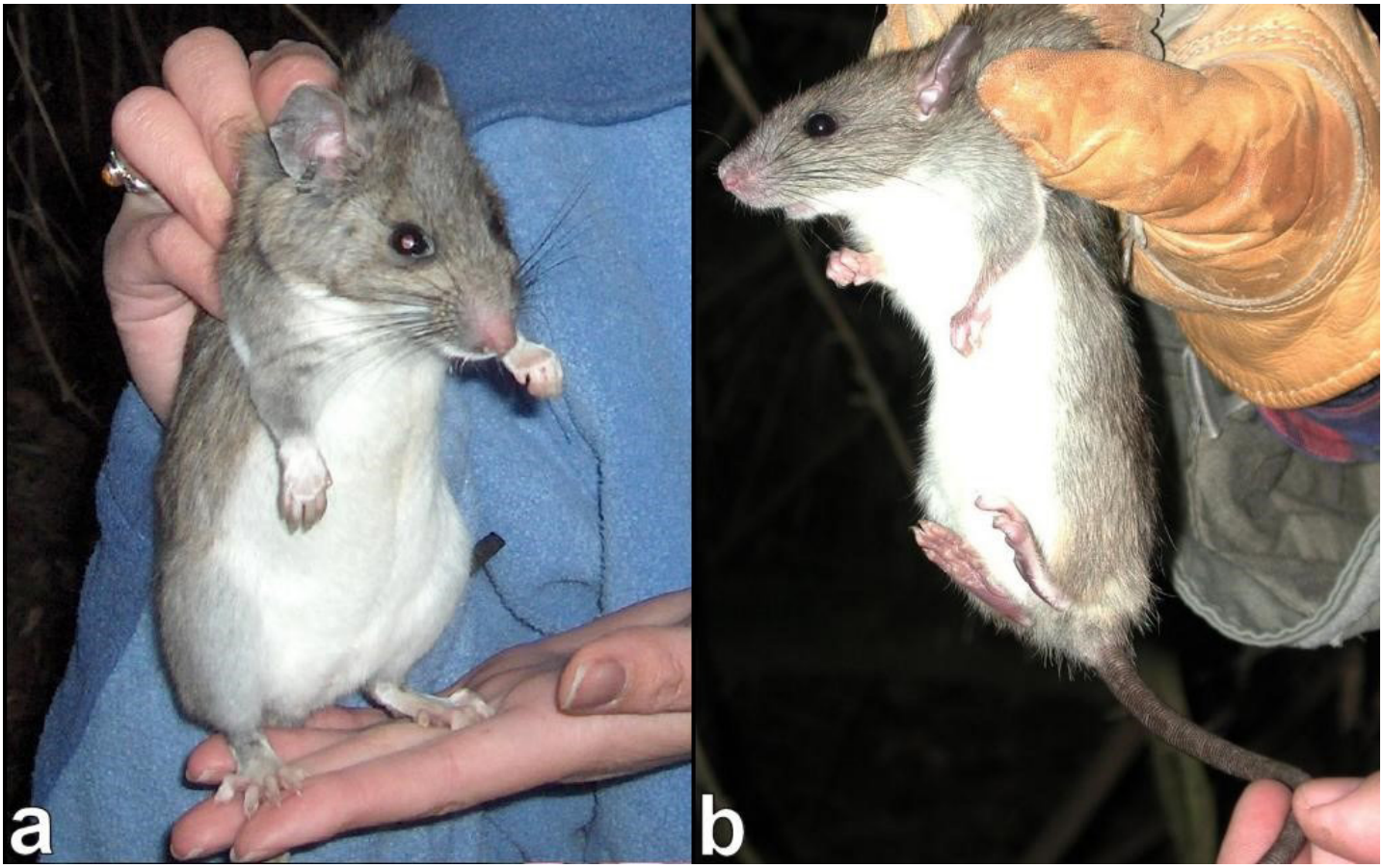

Figure 1. a. Riparian woodrat (*Neotoma fuscipes riparia*); b. Black rat (*Rattus rattus*).

The overall goal of this research was to determine if black rat presence could, either directly or indirectly, be a negative influence on the reproductive success of the riparian woodrat population. The experimental strategy we applied was to compare riparian woodrat abundance and reproductive parameters between two sites, one a control site where both species were present and another site from which black rats were removed.

Materials and methods

Study area. Caswell Memorial State Park comprises approximately 104 ha near Ripon, California in San Joaquin County (Figure 2). The park lies within the floodplain of the Stanislaus River, and elevations range from 9.1 to 13.7 m. Facilities within the park include staff housing, an office building, a maintenance area, two campgrounds, and two day-use picnic areas. Riparian forest is the predominant vegetation community throughout the park. The regional climate includes mild, wet winters and hot, dry summers. Mean high and low temperatures based on data collected in nearby Stockton are 34.6 °C and 15.8 °C in July, and 11.9 °C and 3.1 °C in January. Mean annual precipitation is 354 mm, with most occurring as rain during winter (Western Regional Climate Center 2008).

Our approach for assessing the effects of black rats on riparian woodrats was to identify two sites where both species were relatively abundant. Black rats would then be removed from one site, and woodrat abundance and reproductive success would be compared between the two sites. These sites needed to be sufficiently spatially distinct to ensure minimal probability of animal movement between sites. To identify appropriate sites, exhaustive ground searches were conducted throughout CMSP to locate woodrat sign, particularly stick houses, feces, and runways. Live-trapping also was conducted to verify the presence of woodrats and black rats. The searches and trapping were conducted from September 2001 to August 2002.

In August 2002, two study sites were identified (Figure 3): Site A (Fenceline Trail) the black rat removal site, comprising approximately 11 ha, was located at the northwest edge of CMSP. Site B (Day Use Area) the control site, comprising approximately 9 ha, was about 1 km east of Site A and was located in the central portion of the park.

Dominant tree species at both sites included valley oak, California black walnut (*Juglans californica*), box elder (*Acer negundo*), and willows. The understory was dominated by blue elderberry (*Sambucus mexicana*), California blackberry (*Rubus ursinus*), salmon berry (*Rubus spectabilis*), and stinging nettle (*Urtica diocia*). Additional understory species at Site B included common figs (*Ficus carica*) and wild grape.

Live-trapping. Riparian woodrats and black rats were trapped to assess abundance, determine reproductive

success, and, at Site A, to remove black rats. Trapping was conducted using Tomahawk live-traps (model #201, Tomahawk, Hazelhurst, Wisconsin). Traps were baited with a handful of COB horse feed ('sweet' COB: corn, oats, and barley mixed with molasses). A handful of polyester or cotton batting was placed in the back of each trap for nesting material. Traps were placed in wooden shelters designed to hold one or two traps, and to provide protection from inclement weather, predators, and bait-pilfering birds. Traps were opened and baited beginning about 1 hr prior to sunset and left open for at least 4 hr after sunset. Traps were occasionally left open overnight to increase capture probability.

Trapping was conducted intermittently prior to January 2003 to locate woodrat and black rat populations, and to identify study sites. From January 2003 to September 2004, trapping was conducted weekly with traps being opened for one to two nights each week in fall and winter and two to three nights each week in spring and summer. Traps were placed within 1 m of woodrat houses, by downed debris or dense vegetation actively used by woodrats, and also along active woodrat trails. Additional traps were placed randomly throughout Site A to increase the probability of capturing black rats for removal.

Captured woodrats and black rats were processed using mesh handling bags. Woodrats were handled with 1.25-cm (0.5-in) mesh bags and black rats were handled with 0.6-cm (0.25-in) mesh bags. The bags had an elastic cloth collar that fit securely around the end of a trap. The trap was then opened to allow the animal to enter the bag. Animals were restrained in the bag during processing. Data collected for both species included sex, mass, and age. Each individual received a uniquely numbered ear tag. Woodrats were further marked with passive integrated transponder (PIT) tags inserted subcutaneously, and a tissue sample was collected from an ear with a biopsy punch for genetic analyses. Animals were released at their capture locations. Fecal samples were collected from traps for diet analyses. Mean weights were compared between riparian woodrats and black rats by sex using two-tailed *t*-tests.

Black rat removal. From January 2003 to September 2004, 179 black rats were removed from Site A. Black rats

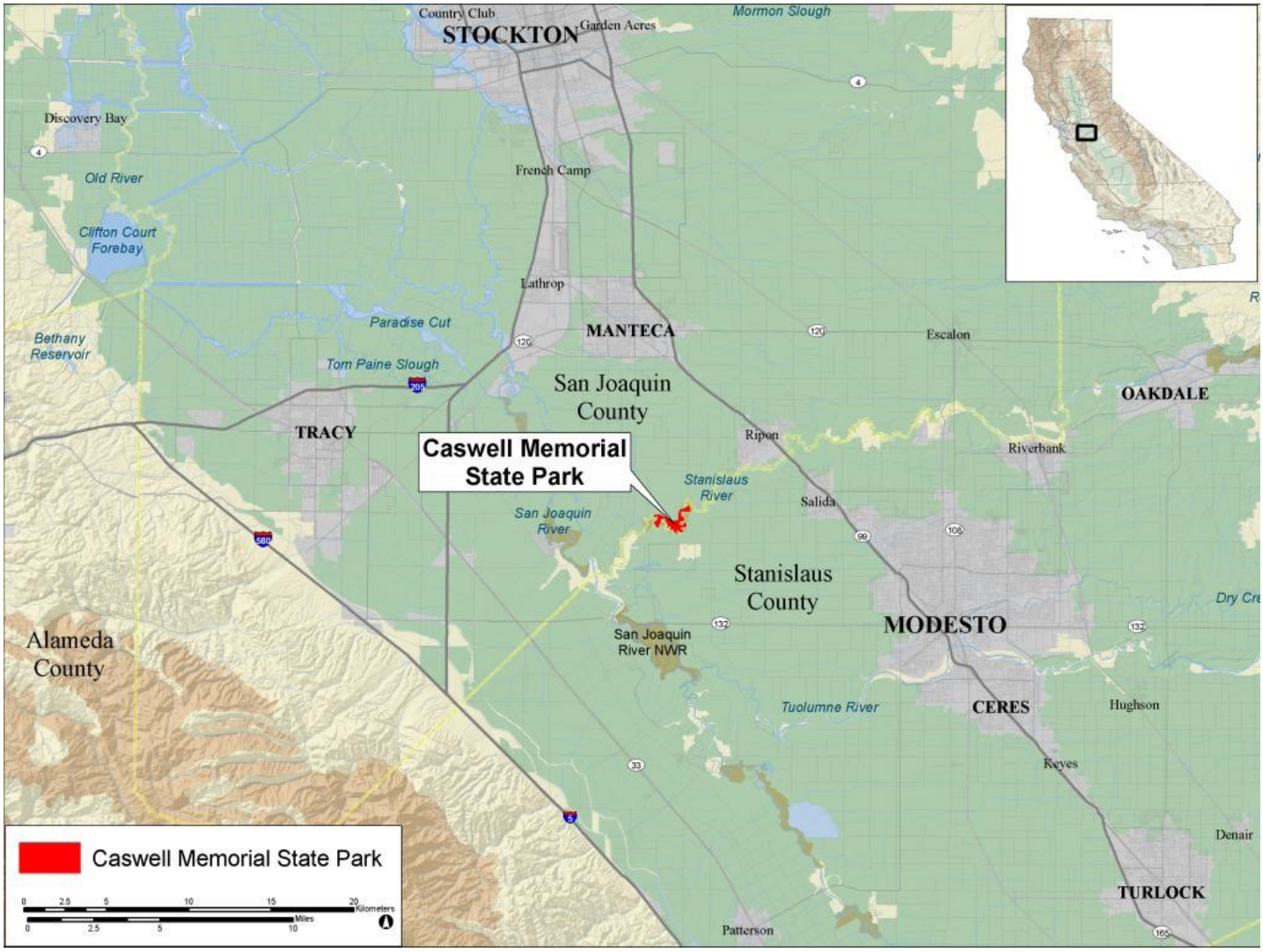

Figure 2. Location of Caswell Memorial State Park, California.

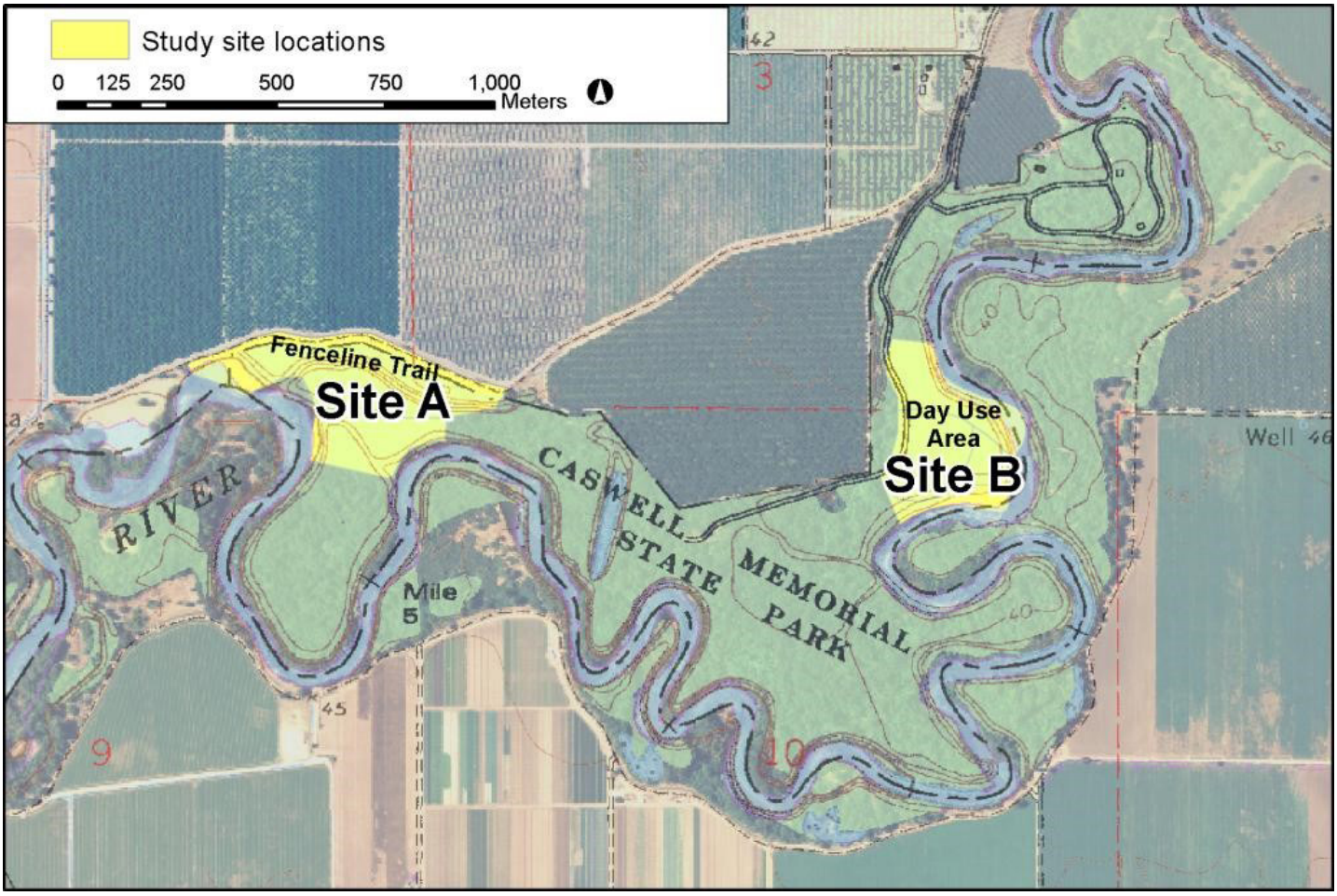

Figure 3. Black rat removal site (A: Fenceline Trail) and control site (B: Day Use Area), Caswell Memorial State Park, California.

were captured during routine live-trapping activities, as described above. Captured black rats were euthanized by injecting an overdose (2 cc) of Pentobarbital Sodium or T-81 (3 cc) into stomach muscles. Carcasses of black rats were collected, labeled, and frozen for future analyses.

Radio telemetry. Radio telemetry was used on some individuals of both species. To minimize risk to the woodrats (required due to their endangered status), radio-collaring techniques and protocols were developed by trapping and collaring non-endangered woodrats (n = 4) at the San Joaquin Experimental Range in Fresno County between 8 May and 24 June, 2002.

At CMSP, radio-collaring was conducted from January 2003 to September 2004. Individuals to be collared were placed in a large glass jar (*ca*. 25 cm tall and 14 cm in diameter) that had attached to a tight-fitting lid a cotton pad that was saturated with three ml of Isoflurane. Once fully sedated and immobile, the animals were removed from the jar for radio-collar attachment. If animals recovered mobility before collaring was completed, up to two additional attempts were made to re-sedate the animal, with an additional 1.5 ml of Isoflurane added on the third attempt. If an animal could not be successfully collared after three attempts, collaring was discontinued and the animal was released. Animals that had been sedated were placed back in traps for at least 20 min to allow them to regain full mobility before being released at the capture site.

Radiocollars (Biotrack Ltd, Dorset, UK) comprised a transmitter affixed to a cable tie-type collar. To prevent snagging, the antenna was wound around the cable tie and secured to it with heat-shrink wrap. The entire unit weighed about 6 g and was < 5% of each animal's body weight. The transmitter signal had a range of 200-600 m.

During the study, 23 woodrats were radio-collared at Site A and 24 were collared at Site B. In addition, 11 black rats were radio-collared at Site B. Radio-collared woodrats and black rats were tracked at least once each week using a telemetry receiver (AVM, Colfax, California and Advanced Telemetry Systems, Isanti, Minnesota) and a two-element "H" antenna (Telonics, Mesa, Arizona). Tracking was conducted primarily during the afternoon, just prior to trap setting. Animals were tracked to their houses or other resting areas. Telemetry locations were used for targeted trapping of female woodrats to assess reproductive condition and to determine whether black rats were using or visiting known woodrat houses or natal dens.

Woodrat Reproductive Success. To assess reproductive success, we examined several reproductive performance

variables for riparian woodrats. We conducted live-trapping on a weekly basis to determine the reproductive status of adult females and to identify young produced by each female. Pregnant females exhibited signs such as a slightly swollen abdomen, a perforate vulva, and pink, loose skin with longitudinal wrinkles in the mammary area. Pregnant females also exhibited a slight loss of weight early in the pregnancy, a gain of >30 g towards the end of pregnancy, and a sudden weight loss upon giving birth (Linsdale and Tevis 1951). These criteria were used to identify reproductive females and estimate the number of litters that each individual produced each year.

Neonatal woodrats remain in their natal dens (usually stick houses) for the first 6-8 weeks of life. Because of the construction and complexity of woodrat houses, accessing nest chambers is difficult, highly invasive, and usually destructive; further, it could place the occupants at risk of injury, abandonment, or predation. Consequently, estimates of reproductive success and the number of young produced by adult females were based on the capture of juveniles following emergence from their natal dens. Juveniles were detected by live-trapping near the houses of females that were determined to have been pregnant and to have produced young. Commonly, multiple traps (2 to 4, or more) were placed near the houses in the hopes of capturing the adult female and multiple young. Individuals weighing less than 200 g at first capture were considered to have been born during the current breeding season. As is common with many small mammals, during their first few months of life, dusky-footed woodrats exhibit a linear growth rate. For a coastal subspecies (*Neotoma fuscipes luciana*), Kelly (1990) aged juvenile woodrats based on their weight at first capture by applying the following formulae:

Males: Age AFC = (weight AFC – 13)/2.167.

Females: Age AFC = (weight AFC – 13.5)/1.828.

where AFC is "At First Capture" and weights are recorded in grams.

One or more of the following criteria were used to assign young to specific adult females:

1. Estimated age of the juvenile corresponded with the estimated date of parturition for the female;

2. Young and an adult female were captured in the same trap or at the same trap station;

3. Young were captured in the same trap or at the same trap station as other known young of a given female;

4. Young were not captured with a female, but were captured at least three times near the house of a lactating female.

In one case, two natal dens were in close proximity to each other and the females associated with these dens had both given birth at about the same time. Thus, the three young captured in this area could not be confidently associated with either female, and therefore 1.5 young were assigned to each female.

Data recorded for each adult female included number of pregnancies and number of emerged young per litter, which generated six variables for analysis.

Number of pregnancies per female (including those where no emerged young were documented).

Litter size: number of emerged young verified for each litter, including litters where no emerged young were recorded (*i. e.*, litter size = 0).

Number of young per female: number of emerged young produced per year (tallied across all litters).

Number of young per pregnancy: number of emerged young for each female divided by the number of pregnancies for that female.

Number of young per litter with young: total number of young for each female divided by the number of litters with young (*i. e.*, litter size ≥ 1) for that female.

Proportion of litters with young: number of litters with emerged young for each female divided by the number of pregnancies for that female.

For each variable, two-way analysis of variance was used to compare mean values between study sites (A and B) and between years (2003 and 2004), and to identify any interaction effect between site and year. Analyses were conducted using Statistica 6.0 (StatSoft, Inc., Tulsa, Oklahoma). Values for proportions of litters with young were transformed prior to analysis using an arcsin transformation (Zar 1984).

Abundance Estimates. Abundance estimates for riparian woodrats and black rats at each site were derived using data from live-trapping and radio telemetry. The minimum number of individuals known to be alive at each site was determined monthly for each species. This estimate was derived by tallying the number of unique individuals captured each month and adding to this the number of radio-collared individuals not captured but known to still be alive and present on the study site (based on telemetry). Individuals captured in non-consecutive months were added to tallies for all intervening months. For each species, mean abundance during the period of black rat removal was compared between sites using a paired-sample *t*-test.

Results

As with most rodents, woodrats and black rats exhibit sexual dimorphism, with males being larger than females. This size dimorphism is more pronounced in adult riparian woodrats (Figure 4). Both sexes are significantly larger for adult riparian woodrats compared to adult black rats (males: $t_{39} = 12.02$, $P < 0.001$; females: $t_{17} = 10.18$, $P < 0.001$; Figure 4).

Woodrat reproductive success. From January 2003 to September 2004, data on reproductive success were collected for 31 adult female riparian woodrats: 13 at Site A and 18 at Site B. At both sites, data were collected for four females in both 2003 and 2004. During the study period, 86 juvenile woodrats were captured, 70 of which (81.4 %) could be associated with individual adult females (42 at Site A, 28 at Site B).

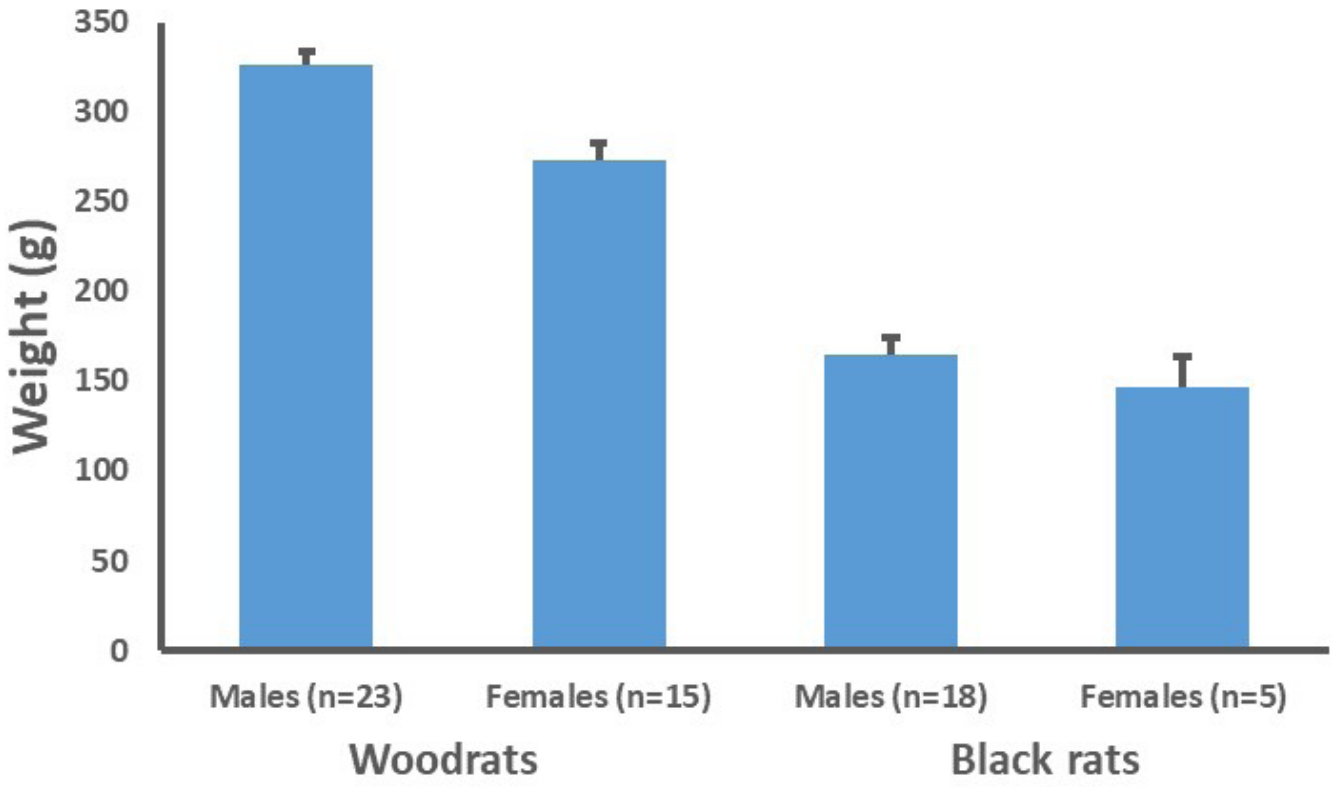

Figure 4. Mean body mass with standard error bars for adult riparian woodrats and black rats at Caswell Memorial State Park, California.

Mean reproductive parameters were summarized by site and year (Table 1). The mean (±SE) number of pregnancies for all female woodrats was 1.59 ± 0.15 (range = 1 to 4) and did not vary between sites ($F_{1,35} > 0.01$, $P = 0.99$) or years ($F_{1,35} = 0.69$, $P = 0.41$), nor was there an interaction between site and year ($F_{1,35} = 0.03$, $P = 0.86$). Mean litter size was almost twice as high at Site A (1.56 ± 0.29) compared to Site B (0.80 ± 0.16), and differed significantly between sites ($F_{1,58} = 5.69$, $P = 0.02$); mean litter size did not differ between years ($F_{1,58} = 1.08$, $P = 0.30$), nor was there an interaction between sites and years ($F_{1,58} = 0.87$, $P = 0.35$). The mean number of young produced by each female was marginally higher ($F_{1,35} = 3.37$, $P = 0.08$) at Site A (2.47 ± 0.61) compared to Site B (1.27 ± 0.30), but did not differ between years ($F_{1,35} = 1.76$, $P = 0.19$), nor was there an interaction between sites and years ($F_{1,35} = 0.24$, $P = 0.63$). The mean number of young per pregnancy for all female woodrats was 1.05 (± 0.17) and did not vary between sites ($F_{1,35} = 2.51$, $P = 0.12$) or years ($F_{1,35} = 0.96$, $P = 0.33$), nor was there an interaction between site and year ($F_{1,35} = 0.20$, $P = 0.66$). However, consistent with observed values for the other parameters, the mean number trended higher at Site A and in 2003. The mean number of young for litters with emerged young was significantly higher ($F_{1,35} = 8.18$, $P = 0.01$) at Site A (2.39 ± 0.31) compared to Site B (1.51 ± 0.18), but did not differ between years ($F_{1,35} = 0.15$, $P = 0.70$). However, there was a marginally significant interaction between site and year ($F_{1,35} = 3.13$, $P = 0.09$), with the mean number of young for litters with emerged young increasing on Site A from 2003 to 2004 but decreasing at Site B (Figure 5). Finally, the mean proportion of litters with emerged young was 55.1 ± 7.2 % and did not differ between sites ($F_{1,35} = 0.77$, $P = 0.39$) nor was there an interaction between site and year ($F_{1,35} = 0.27$, $P = 0.61$). However, the mean proportion was marginally higher ($F_{1,35} = 3.04$, $P = 0.09$) in 2003 (67.6 ± 10.1 %) compared to 2004 (44.4 ± 9.8 %).

Abundance estimates. Monthly estimates of the minimum number of woodrats known to be present ranged from 4 to 34 at Site A and 19 to 33 at Site B (Figure 6). Mean monthly abundance of woodrats during the 21 months of black rat removal was higher ($t_{1,20} = -4.07$, $P > 0.01$) at Site B (27.2 ± 1.1) compared to Site A (21.1 ± 1.6). However, the patterns of abundance differed between the two study sites. Both populations exhibited an increase during May to July 2003, which likely was associated with reproduction and the presence of emerged young. At Site A, abundance decreased again in August 2003 and remained at a lower level until increasing again in April 2004 in association with annual reproduction. Abundance then declined from July 2004 until the end of the study in September 2004. Conversely, after increasing in spring 2003, abundance at Site B remained at a consistently high level through June 2004, after which abundance declined monthly through the end of the study, similar to that observed at Site A.

Black rat abundance also differed between the two study sites (Figure 6). At Site B, monthly estimates of the minimum number known alive ranged from 0 to 28, peaking each spring and summer coincident with annual reproduc-

Table 1. Reproductive parameters (mean and standard error) by study site and year for female riparian woodrats at Caswell Memorial State Park, California, from January 2003 to September 2004.

Site[1]/ Year	No. females	Pregnancies per female	Litter size	Young per female	Young per pregnancy	Young per litters with young	Percent litters with young
Site A	17	1.6 (0.2)	1.6 (0.3)	2.5 (0.6)	1.4 (0.3)	2.4 (0.3)	61.3 (0.1)
Site B	22	1.6 (0.2)	0.8 (0.2)	1.3 (0.3)	0.8 (0.2)	1.5 (0.2)	50.4 (0.1)
2003	18	1.7 (0.3)	1.3 (0.2)	2.3 (0.5)	1.2 (0.2)	1.9 (0.2)	67.6 (0.1)
2004	21	1.5 (0.2)	0.9 (0.2)	1.4 (0.4)	0.9 (0.3)	1.9 (0.3)	44.4 (0.1)
Total	39	1.6 (0.2)	1.1 (0.2)	1.8 (0.3)	1.1 (0.2)	1.9 (0.2)	55.1 (0.1)

[1] Site A. Fenceline Trail, black rats removed. Site B. Day Use Area, black rats not removed.

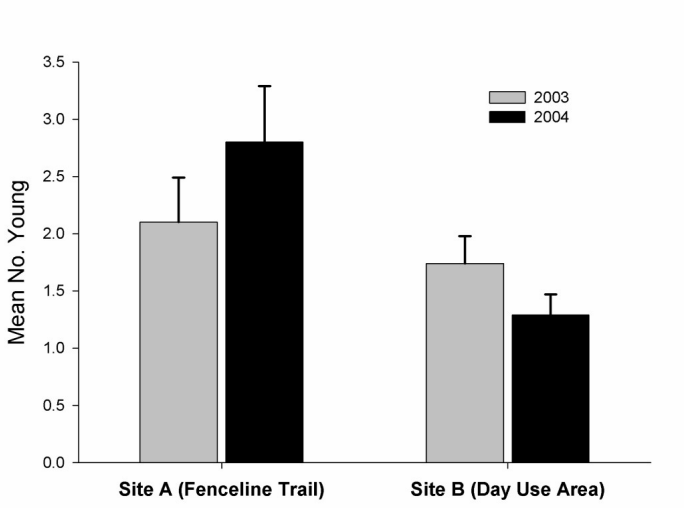

Figure 5. Mean number of young with standard error bars for litters with emerged young on the black rat removal site (A: Fenceline Trail) and control site (B: Day Use Area), Caswell Memorial State Park, California, from January 2003 to September 2004.

tion. Interestingly and for unknown reasons, the population declined precipitously after July 2004 and no black rats were captured at Site B in the last month of the study. At Site A, abundance estimates ranged from 3 to 28. Black rat removals began in January 2003, and abundance declined markedly by April 2003. Abundance increased again during May-July 2003, probably as a result of reproduction, but then plummeted in August and stayed at a relatively low level for the remainder of the study. After July 2003, abundance estimates remained at 10 or lower. This suggests that removal efforts were successful in markedly reducing black rat abundance at Site A. Mean monthly abundance during the 21-month removal effort was significantly higher ($t_{1,20} = -3.33$, $P > 0.01$) at Site B (15.9 ± 1.5) compared to Site A (9.6 ± 1.7).

Discussion

Interactions between endangered riparian woodrats and non-native black rats at Caswell Memorial State Park were investigated for a 37-month period (September 2001 to September 2004), with the response of woodrats to black rat removal being investigated for 21 of those months (January 2003 to September 2004). The removal of black rats from Site A (Fenceline Trail) appeared successful in reducing black rat abundance at that site. This was a consequence of several months of trapping, but from late summer 2003 until the end of the study, black rat abundance remained consistently lower at Site A than at Site B (Day Use Area).

Of six parameters of reproductive success examined for woodrats, two (mean litter size and mean number of young per litter-with-young) were significantly higher and another (mean number of young per female) was marginally higher on the black rat removal site. Furthermore, the mean number of young in litters with young increased substantially between years at Site A, but exhibited a concomitant decrease at Site B, from which black rats were not removed. Collectively, these results suggest that woodrat reproductive success was higher at Site A. Although the positive response at Site A cannot be conclusively attributed to the removal of black rats, the implied correlation is compelling.

Reduced black rat abundance may have benefited woodrat reproductive success in several ways. Through exploitative competition, black rats may have been reducing the availability of resources (*e.g.*, food, houses) to woodrats. Nutritionally stressed woodrats likely would produce fewer offspring, either through pregnancy failure, reduced litter size, or reduced juvenile survival. If woodrats were being displaced from houses, reproductive success could be lowered through exposure of young to the elements (*e.g.*, precipitation, cool nighttime temperatures) or increased predation rates due to a lack of adequate shelter. However, we have no evidence to suggest that black rats can exploit resources essential to woodrat survival. Adult woodrats are larger than adult black rats, and woodrats are known to aggressively defend resources (Linsdale and Tevis 1951), so we believe that in one-on-one interspecific encounters, adult woodrats (of either sex) would dominate adult black rats.

Interference competition from black rats could also have reduced woodrat reproductive success. Although we did not produce any data in support of this, black rats have the potential to prey on woodrat young, thereby causing direct mortality; they could prey on nestling young while the mother is out foraging or they could potentially prey

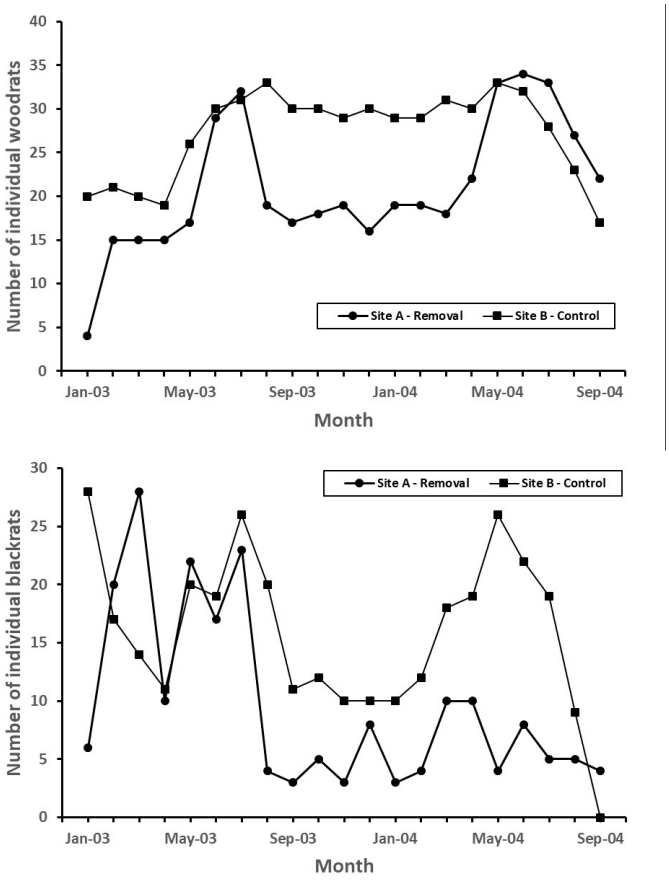

Figure 6. Minimum number of riparian woodrats (top) and black rats (bottom) known to be alive on two study sites—Site A - black rat removal (Fenceline Trail) and Site B - control (Day Use Area)—at Caswell Memorial State Park, California, from January 2003 to September 2004.

on or harass and drive off emergent young. Even if direct predation is not occurring, harassment by black rats or even just their presence, especially if the black rat population density was as high or higher than the woodrat population, could negatively affect woodrat reproductive success. For example, if woodrats were spending inordinate amounts of time repelling black rats, then less time would have been available for attending young or feeding to maintain a sufficient nutritional plane to rear young (*e.g.*, energy for lactation). In southeastern Australia, Stokes *et al.* (2009) concluded that black rats were reducing reproductive success of native bush rats (*Rattus fuscipes*), apparently through interference competition with juvenile bush rats.

Although black rat and woodrat densities were not markedly different at the Day Use Area (Site B), long-term trapping data for CMSP indicates that black rat density can be significantly higher than woodrat density. Furthermore, the presence of black rats could constitute an environmental stressor for woodrats. Chronically elevated glucocorticoid steroid hormones associated with persistent stressors can produce a suite of deleterious physiological effects including reproductive suppression (Sapolsky *et al.* 2000). Thus, removal of black rats could have reduced competitive pressure and stress experienced by woodrats.

The effect of black rat removal on the abundance of riparian woodrats was more equivocal. Despite the reduction in black rat abundance at Site A, woodrat abundance at this site was typically lower than that at Site B. Not until the last five months of the study did woodrat abundance on Site A equal and then exceed that at Site B.

It is possible that the black rat population at CMSP is a consequence of it being a state park, and in particular because it has a campground as well as day use areas. From ESRP trapping results through 2005, black rats do appear to be more common in the campground area but they are also quite common in other areas of the park, including relatively isolated areas such as along Fenceline Trail. Further, black rats are known to be common in other riparian areas throughout the San Joaquin Valley. Here we provide just two examples of their pervasiveness in natural areas. Since its initiation in 2002, black rats have been frequent captures in the riparian brush rabbit (RBR, *Sylvilagus bachmani riparius*) recovery implementation program at the San Joaquin River National Wildlife Refuge and other nearby RBR release sites (Faith Ranch, Buffington Tract; latter is across the Stanislaus River from CMSP). Also, between 2004 and 2008, an average of 124 black rats were captured and removed annually from the RBR breeding pens near Lodi, an area that has very little human disturbance or anthropogenic food sources (except perhaps nearby croplands, although little appears to be known about the extent of use of croplands by *Rattus* in California).

The results of this investigation are not definitive. Certain intrinsic and extrinsic attributes of the data may have precluded detection of differences between our sites, and possibly confounded our results. For example, it is possible that a positive response by woodrats might have become more pronounced with a longer period of black rat removal. The removal in this study was conducted for just 21 months. A cause-and-effect relationship might have been more strongly supported with a longer study. Also, there may have been environmental differences (e.g., habitat suitability, food availability) between sites that might have affected differences in the response by woodrats to black rat removal. Annual environmental variation and even stochastic demographic variation may have contributed to this effect.

All of the above would have rendered the detection of differences between the two study sites more difficult. Despite this, the results did provide strong evidence of certain trends, most of which indicated that the removal of black rats conferred a benefit to woodrats. However, two cautions are in order. Autocorrelation among some of the reproductive parameters could have inflated the number of parameters exhibiting differences. Also, a marked increase in the abundance of raccoons, a potential predator of woodrats, in spring 2004 at Site B could have contributed to the decrease in woodrat reproductive success between 2003 and 2004 at that site.

Black rats are native to the Indian sub-continent but have been unintentionally spread by humans to all continents except Antarctica. Due to immense ecological plasticity, they can occupy a diversity of habitats and consume a wide range of foods consisting of plants, fungi, and animals. Consequently, black rats have caused or contributed to the extinction of various species of birds, small mammals, reptiles, invertebrates, and plants (Invasive Species Specialist Group 2008). Catastrophic declines of many bird populations attributable to black and Norway rats (*Rattus norvegicus*) are well documented (*e.g.*, Atkinson 1985; Thibault *et al.* 2002; Major *et al.* 2006; Invasive Species Specialist Group 2008).

Black rats have adversely impacted native rodents, including rare species, in other ecosystems worldwide, particularly insular ones. In the Galapagos Islands, black rats either directly caused or contributed to the extinctions of three of seven endemic rice rat species (*Oryzomys* spp. and *Nesoryzomys* spp.). Of the four extant species, the Santiago rice rat (*N. swarthi*) was presumed extinct, but small numbers were rediscovered on Santiago Island in 1997 (Dowler et al. 2000). Removals of black rats on this island significantly slowed the rate of population decline of rice rats, primarily through increased survival of female rice rats (Harris and Macdonald 2007). Similarly, populations of rare birds and snakes increased on Antiguan islands following the removal of black rats (Daltry *et al.* 2012). Finally, as mentioned previously, black rats have also been implicated in the decline of the endangered Key Largo woodrat in Florida (Sasso and Gaines 2002).

Our study strongly suggests that black rats also can have impacts in a non-insular setting. Similarly, black rats were found to be adversely affecting the native bush rat in rainforest habitat in southeastern Australia (Stokes *et al.* 2009). Bush rat populations increased significantly following removal of black rats, and the latter did not re-establish after removal as the increase in bush rats, particularly adult females, apparently shifted the competitive advantage to bush rats.

The results of this investigation indicate that black rats may have adverse impacts on riparian woodrat populations. The woodrat population at CMSP had been recognized as the only extant population for this subspecies (USFWS 1998). While there is little recent information on woodrat abundance in the park, there is increased concern about the status of the population. A recent ground search (~2 hours on April 18, 2024) for woodrat sign (houses, vegetation clippings, droppings) could not confirm woodrat presence. However, an adult riparian woodrat was captured on video during vegetation management activities on October 29, 2024 (C. Bradley, California State Parks, pers. comm.). In March 2003, a putative riparian woodrat was captured at the San Joaquin River National Wildlife Refuge (SJRNWR) about 8 km south of CMSP and over 30 additional individuals have been captured since (CSU Stanislaus, Endangered Species Recovery Program, unpublished data). Genetic analyses of samples from these individuals indicated that similar to the woodrats at CMSP, the ones at SJRNWR include an admixture of genes from *N. fuscipes* and *N. macrotis* (Matocq *et al.* 2012). However, even if the SJNWR population is classified as the riparian woodrat, it would constitute only a second population for the subspecies, and having just two recognized populations would still leave this subspecies with a very restricted distribution and extremely vulnerable to catastrophic events (*e.g.*, wildfire, floods, disease). Thus, any reasonable efforts should be employed to conserve these populations. These efforts could include black rat control or removal.

Removing black rats on a sustained, long-term basis would no doubt be challenging, and complete removal is probably impossible. Black rats exhibit high reproductive rates and rapid dispersal and in central California they occur in urban areas and in habitats having dense vegetation, especially riparian habitat. Thus, accessing black rats is difficult and removed rats might be quickly replaced through reproduction or immigration. Furthermore, unlike some other locations where large-scale broadcasting of rodenticide-laced baits has been used to reduce or eliminate black rats (*e.g.*, Howald *et al.* 2005), impacts to non-target species, as well as the presence of endangered woodrats, negates use of this strategy. Thus, live-trapping and euthanizing black rats currently is the only practical removal method that minimizes risk to woodrats. However, this strategy is labor intensive and therefore expensive.

Another potential strategy to help control black rat abundance might be the reintroduction of ringtails (*Bassariscus astutus*) to CMSP and other riparian areas of the northern San Joaquin Valley. Recent research in the Sacramento Valley has indicated that ringtails prey heavily on black rats (D. Wyatt, Sacramento City College, pers. comm). Provided that ringtail reintroduction would not adversely impact riparian woodrat and riparian brush rabbit populations, this may be a useful strategy to consider. We would expect ringtails to prey on woodrats, and possibly also brush rabbits, but if they differentially prey on black rats, that could result in a competitive advantage for woodrats (and brush rabbits) over black rats.

Finally, one strategy that might be practical and cost-efficient is to identify a set of "core areas" that encompass optimal habitat for woodrats and then focus black rat removal efforts on only those areas. Each area should be sufficiently large to support a sizeable woodrat population, but also sufficiently small such that black rat numbers could be effectively reduced via a standardized live-trapping program, especially during the woodrat breeding season, along established trap lines or trapping stations. Depending on habitat parameters (woodrats can reach high densities under optimal habitat conditions), an area of 10-20 ha might be sufficient to achieve these objectives. Valuable information as well as cost efficacy could be gained by also using these trapping efforts to monitor the woodrat population.

Recommendations. Based on our results, we offer the following recommendations.

A minimum of two core areas of 10 to 20 ha in size, with high quality habitat and high density woodrat populations, should be identified at CMSP.

A black rat removal program should be implemented within these core areas.

Conducting removals multiple times per year would be most effective, but minimally removals should be conducted in the 3 months (January to March) prior to the woodrat breeding season to minimize impacts by black rats on woodrat reproductive success.

Explore the use of emerging fertility control methods for black rats. Such methods would need to be specific to black rats to avoid impacting woodrats.

Surveys should be conducted in locations other than CMSP to identify additional riparian woodrat populations.

If black rats are present, as expected, in any newly identified riparian woodrat populations, then a removal program, similar to the one proposed at CMSP, should also be conducted in those locations.

In all locations with riparian woodrats, a concerted effort should be made to remove or reduce anthropogenic sources of food and shelter for black rats.

Until additional riparian woodrat populations are found, a captive colony of riparian woodrats should be established, particularly given the myriad threats (*e.g.*, black rats, flooding, wildfire, disease, predators) to the CMSP population.

In addition to serving as insurance against a catastrophic event at CMSP, a captive colony could be used to breed riparian woodrats for introduction into areas with suitable vacant habitat.

The reintroduction of ringtails to CMSP and other riparian areas of the northern San Joaquin Valley should be investigated further. Successful reintroduction of ringtails could not only benefit riparian woodrats and riparian brush rabbit, but it would also restore a semblance of the more complete riparian community that existed in the 19th and early 20th centuries.

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Eavesdropping on bats in Peninsular Thailand: a trial application of automated recorders to monitor habitat changes

Grace Rui-Tong Yu[1], Ariya Dejtaradol[2], Yingyod Lapwong[2], Sunate Karapan[3], Pipat Soisook[4], Chia-Yun Lee[5], Mao-Ning Tuanmu[5], Hon-Tsen Yu[6] *

[1] Department of Biology, University of British Columbia Okanagan, Kelowna. British Columbia, Canada V1V 1V7. Email: grace.yu945@gmail.com (GR-TY). Current address: Kang-Chiao International School, Xin-Dian District, New Taipei City. Taiwan ROC 231.
[2] Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai. Songkhla, Thailand 90110. Email: ariya.d@psu.ac.th (AD), yingyod.l@psu.ac.th (YL).
[3] Halabala Wildlife Research Station, Wildlife Research Division, Wildlife Conservation Bureau, Department of National Parks, Wildlife and Plant Conservation. Narathiwat, Thailand, 90160. Email: karapann@gmail.com (SK).
[4] Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Hat Yai. Songkhla, Thailand, 90110. Email: pipat.s@psu.ac.th (PS).
[5] Biodiversity Research Center, Academia Sinica, Taipei. Taiwan, ROC 115. Email: janiceli0918@gmail.com (C-YL), mntuanmu@gate.sinica.edu.tw (M-NT).
[6] Department of Life Science, National Taiwan University, Taipei. Taiwan, ROC 106. Email: ayu@ntu.edu.tw (H-TY).
*Corresponding author: ayu@ntu.edu.tw.

We deployed automated bat recorders for one week in the southern part of Peninsular Thailand in an attempt to monitor bat species diversity and activities. Two sites were chosen: one on a forested slope adjacent to urban development, the campus of Prince of Songkla University (PSU), and the other in a natural tropical rainforest, the Hala-Bala Wildlife Sanctuary (Bala). From PSU, we analyzed 9,744 5s time windows of recordings that were obtained from the dry season (June 2023) and the wet season (October 2023); from Bala, we analyzed 4,692 5s time windows in the wet season (October 2023). Among a total of 14,436 time windows, we detected bat acoustic signals in 1986 (13.8 %) representing 10 species of bat: eight species at PSU and six species at Bala. The recordings permitted analyses of diel activity patterns for the four species with the most acoustic records, as well as estimates of relative species abundance in accordance with forest type and season. Our results demonstrate that using automated bat recorders can help unravel bat diversity, activity patterns, and the potential for interspecific interactions. Nonetheless, independent efforts to collect and verify acoustic signals by catching and observing live bats are needed to ensure accurate species identification.

Se utilizaron grabadoras automáticas de llamados de murciélagos durante una semana en la parte sur de la península de Tailandia en un intento de monitorear la diversidad y las actividades de las especies. Se eligieron dos sitios: uno en una ladera boscosa adyacente al desarrollo urbano, el campus de la Prince of Songkla University (PSU), y el otro en una selva tropical natural, el Santuario de Vida Silvestre Hala-Bala (Bala). De PSU, analizamos 9,744 ventanas de tiempo de 5 s de grabaciones que se obtuvieron de la estación seca (junio de 2023) y la estación lluviosa (octubre de 2023); de Bala, analizamos 4,692 secciones de tiempo de 5 s en la estación lluviosa (octubre de 2023). Entre un total de 14,436 secciones de tiempo, detectamos señales acústicas de murciélagos en 1986 (13.8 %) que representan 10 especies de murciélagos: ocho especies en PSU y seis especies en Bala. Las grabaciones permitieron realizar análisis de los patrones de actividad diurna de las cuatro especies con más registros acústicos, así como estimaciones de la abundancia relativa de especies según el tipo de bosque y la estación. Nuestros resultados demuestran que el uso de grabadoras automáticas de murciélagos puede ayudar a desentrañar la diversidad de murciélagos, los patrones de actividad y el potencial de interacciones interespecíficas. No obstante, se necesitan esfuerzos independientes para recopilar y verificar señales acústicas mediante la captura y observación de murciélagos vivos para garantizar una identificación precisa de las especies.

Keywords: Acoustic signals; automated recorder; bat; diel activity pattern; habitat monitor; peninsular Thailand.

Introduction

Sensor technologies, including automatic bat recorders, are increasingly used for passive monitoring of biodiversity and for ecological surveillance (Sethi *et al.* 2020; Yoh *et al.* 2022). The prolonged and consecutive nature of such recordings offers insights into animal activity at a much greater level of detail than previously possible, especially for echolocating bats, which are primarily nocturnal and often secretive. Furthermore, high-frequency echolocating sounds emitted by bats residing in a particular habitat should be identifiable and distinguishable from those of bat assemblages (sonotypes and their relative abundances) in other habitat types. Bat ultrasonic signals should be amenable to this purpose because bat foraging behaviors are known to adapt to prey and habitat types (Denzinger *et al.* 2018; Yoh *et al.* 2022). In Thailand, bat calls have been analyzed to the species level (Hugh *et al.* 2011; Ith *et al.* 2011) and these data could serve as a point of reference for future studies. However, no previous studies have employed automated recorders to monitor diversity or estimate species distributions.

Peninsular Thailand is of particular interest to biogeographers because the region is situated on the northern

part of the Thai-Malay Peninsula and represents a transition zone of biodiversity between the Indochinese and Sundaic faunas. Many zoogeographical studies have identified the Isthmus of Kra as a major boundary line between the two faunas, while the Kangar-Pattani Line lying *ca.* 500 km to the south distinctly separates northern and southern plant communities (Lohman *et al.* 2011). Some researchers propose that the biogeographical divergence in Peninsular Thailand resulted from repeated fluctuations in sea level over the last 5 million years rather than permanent physical barriers, such as mountains and rivers (Woodruff and Turner 2009; Woodruff 2010; Li and Li 2018). Additionally, studies focusing on avifauna have shown that changes in bird species composition near the Isthmus of Kra are linked to shifts in forest type driven by climatic factors (Hughes *et al.* 2003; Dejtaradol *et al.* 2015). A study of amphibians identified the area between the Isthmus of Kra and the Kangar-Pattani Line as a distinct biogeographic subregion called South Tenasserim (Poyarkov *et al.* 2021). Consequently, Peninsular Thailand is characterized as a broad biogeographical transition zone that has been shaped by multiple episodes of separation rather than a singular, sharp delineation like that represented by the Wallace Line (Hinckley *et al.* 2023).

Fourteen provinces are administered in Peninsular Thailand and are known collectively as the Region of Southern Thailand, with a combined area of 70,714 km^2. The western part of the region features steep coastlines; on the eastern side alluvial plains predominate. The largest plain, located in Surat Thani, is formed by two rivers, the Tapi and the Phum Duang, with a total catchment of more than 8000 km^2. Smaller rivers either empty into the Gulf of Thailand (*e. g.*, the Pattani and the Saiburi) or into the Andaman Sea (*e. g.,* the Krabi and the Trang). Additionally, Songkhla Lake (1,040 km^2), the largest lake in Thailand, is a conspicuous feature and wildlife habitat in this region.

Peninsular Thailand is longitudinally divided by the southern section of the Tenasserim Range, resulting in two narrow coastal plains that experience distinct climatic conditions. The Phuket Subrange extends from the Isthmus of Kra down to Phuket Island. Approximately 100 kilometers to the east lies the Nakhon Si Thammarat, or Banthad Subrange, which begins at Phangan Island and continues southward to Songkhla Province, where it connects with the Titiwangsa Range. The majority of Peninsular Thailand belongs to the Tenasserim-South Thailand semi-evergreen rain forest ecoregion. In the adjacent region, the Peninsular Malaysian rain forest and montane rain forest ecoregions extend into southernmost Thailand (Olson *et al.* 2001). Today large tracts of rubber and oil palm plantations have replaced the natural forests and dominated the once forested landscape.

Thailand, as a whole, has lost 20 % of its forest cover over the last 40 years, from 53 % in 1961 to 33 % in 2000 (Bumrungsri *et al.* 2006), a loss of *ca*. 0.5 % per year. This is largely because rubber trees were introduced as a cash crop in the early 1900s and replaced native tree species. In the Region of Southern Thailand, large areas of rainforest have been converted to rubber plantations. By 1992, 25 % of the land area of Southern Thailand was occupied by rubber plantations and just 18 % remained forested. Oil palm cultivation was introduced in the 1980s (Dallinger 2011), either replacing existing rubber plantations or expanding into newly-cleared forested areas. Today, both rubber and oil palm trees dominate the landscape.

Peninsular Thailand is rich in bat diversity, containing at least 87 species of bats in eight families using laryngeal echolocation (Emballonuridae, Megadermatidae, Rhinolophidae, Hipposideridae, Vespertilionidae, Miniopteridae, Molossidae, and Nycteridae; Karapan *et al.* 2023). A detailed study of bats conducted by Phommexay *et al.* (2011) over the course of seven months contrasted bat diversity in natural rainforests with that in rubber plantations in southern Peninsular Thailand (Songkhla Province and Phatthalung Province). That study revealed a depauperate bat fauna in rubber plantations (26 species in rainforests as compared to 13 species in rubber plantations), suggesting that recent changes in land use have negatively impacted bat diversity.

We installed automated bat recorders in two forested areas in Southern Thailand, aiming to test their feasibility in monitoring the impact of the habitat changes on bat diversity caused by deforestation and cultivation of economically-important plants. Specifically, we chose a natural reserve area (Bala site) in which original forests remain intact and a site (PSU) where rubber trees had been planted but were later abandoned and ceased to be managed as plantations, permitting secondary forests to regrow. Bat acoustic signals were recorded and analyzed to examine the differences in bat diversity, behavior and activity patterns in relation to the differences in habitat and land use between these sites.

Materials and methods

Study sites. Two sites were selected for recording (Figure 1): 1) two recorders were installed at the Prince of Songkla University (PSU) site one recorder was placed at the Hala-Bala Wildlife Sanctuary (Bala) site(Appedix 1: Plate 1 and 2). At PSU, the recorders were set on a forested slope of Kho-Hong Hill adjacent to the eastern side of the campus. Large tracts of rubber plantations used to exist on this slope after the native forest was cleared. In 1976-1980, PSU procured this land for conservation purposes, permitting it to revert to natural habitat through succession (Bumrungsri *et al.* 2006). Major native trees include *Schima wallichii*, *Castanopsis schefferiana*, *Memecylon edule*, *Diospyros frutescenes*, *Diplospora malaccensis* (Bumrungsri *et al.* 2006); the most common understory species (scrubs and treelets) include *Ixora javanicai*, *Psederanthemum graciliflorum*, *Mesua kunstleri* (Maxwell 2006). One recorder (PSU1) was set in a spot where abandoned rubber trees are still conspicuous but where undergrowth and some native trees have returned. The other recorder (PSU2) was set closer to the ridge where there was no evidence of rubber trees

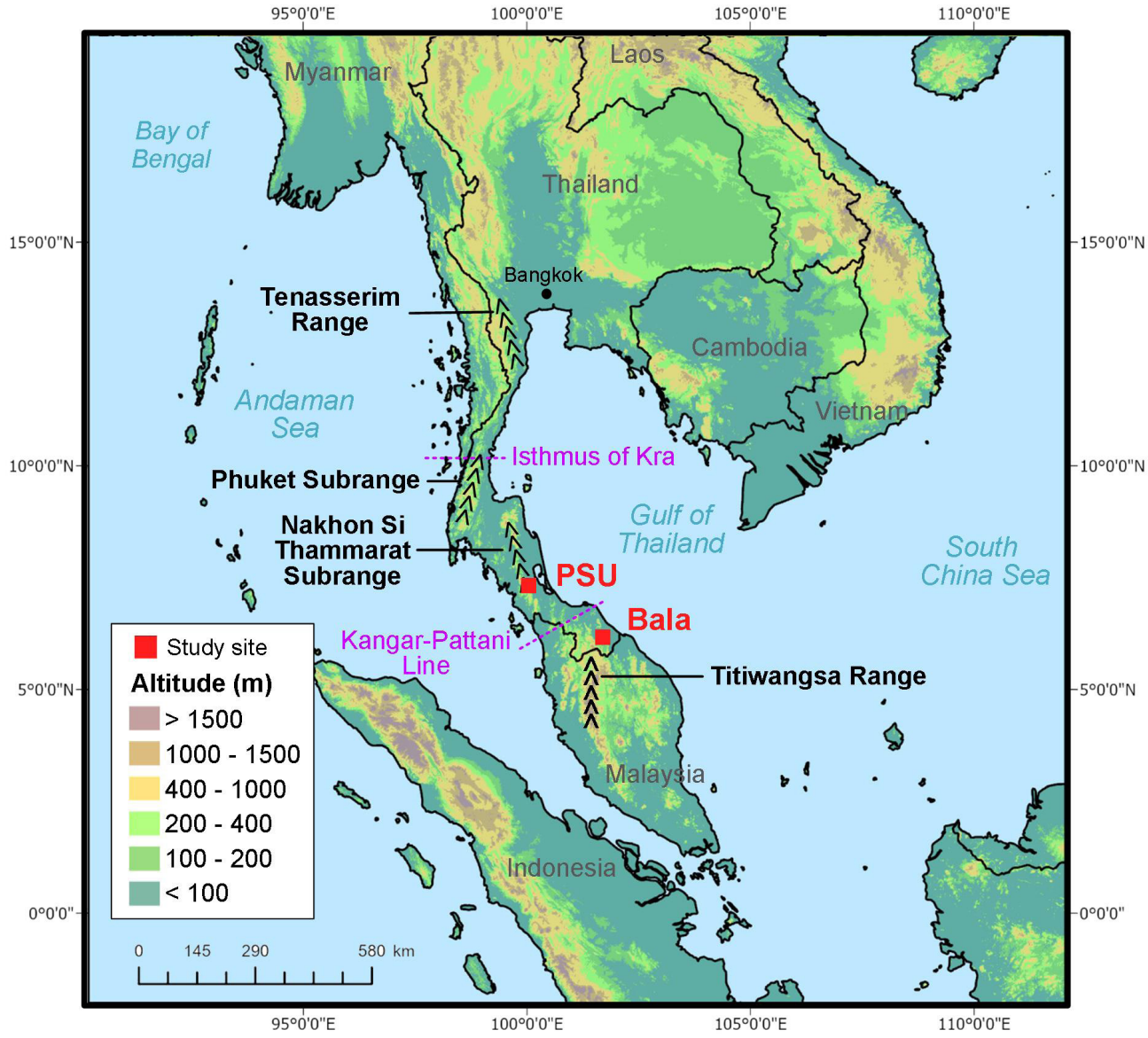

Figure 1. Map of Peninsular Thailand showing the locations of automated bat recorders installed. PSU: Prince of Songkla University; Bala: Halabala Wildlife Sanctuary. Isthmus of Kra, Kangar-Pattani Line, and major mountain subranges and ranges are also shown. The altitude data are taken every 7.5-arc-second spatial resolution from the Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010).

and where much denser undergrowth was observed. At Bala, a single recorder was set in the vicinity of a stream in a mature lowland dipterocarp forest near the research station compound (canopy height *ca*. 40 to 60 m, composed of Malaysian flora; Kitamura *et al.* 2011). The lower level of the forest at this site is covered by dense undergrowth. The only light penetrating is through occasional gaps in the canopy. Both sites are of typical tropical climate with alternating wet-dry seasons. Previously recorded data (1981 to 2010) indicate that average monthly rainfall for the month of October (early wet season) was about 2 to 2.5 times that for the month of June (late dry season), 257/100 mm at PSU and 254/123 mm at Bala (Thai Meterological Department, https://www.tmd.go.th/en).

Recording setup. We conducted audio recordings during two periods—June 2023 and October 2023—corresponding roughly to the late dry season and the early wet season in Southern Thailand, respectively. At Bala, we recorded in both seasons but recording files of the dry season were lost due to a malfunction of the recorder. Song Meter Mini Bat 2 AA recorders were used in this study (Wildlife Acoustic, Maryland, MA, USA). Each recorder was mounted on a tree at a height of 1.5 to 1.8 m aboveground and data disks were collected at the end of the month. The recorders' sampling frequencies were set to 384 kHz capturing a one-minute file every fifteen minutes from evening to dawn (16:00 to 7:00 hr).

Acoustic analysis. Sound files (.wav) were analyzed using Kaleidoscope Lite Analysis software (version 5.6.2; Wildlife Acoustic, Maryland, MA, USA). Each one-minute recording was segmented into twelve 5-second windows for detailed analysis, after which bat calls were manually screened and

labeled by one of us (Grace Rui-Tong Yu), after which they were identified to species by a local bat specialist (Pipat Soisok) who has extensive experience capturing and recording bat species in the region. Four characteristic measurements were used for identification: frequency of maximal energy, start frequency, terminal frequency, and call duration. All call files were deposited in the Natural History Museum of Prince Songkla University and are available upon request. We treated windows that contained bat calls involving at least three repetitions as a passing (fly by) event. Since we could not ascertain whether all the calls in one window were produced by one bat or several bats, we quantified them as one passing. Windows with calls that clearly overlapped or contained different signal structures were counted as separate passings. To enhance the detection of legitimate bat calls, the software's signal detection parameters were set as follows: frequency range 12 kHz to 180 kHz, pulse length 1 ms to 80 ms, maximum interpulse gap 500 ms, and a minimum of 3 pulses per detection. Files not fulfilling these requirements were labeled as "noise" files. To prevent mislabeling files that might contain legitimate bat calls, we randomly selected files labeled as "noise" for re-screenings, but this reassessment did not retrieve any legitimate bat calls that had been labeled as "noise". Therefore, all noise files were purged without further analysis. Spectrograms (.png) were made by BatSound (version; Pettersson Elektronik AB, Uppsala, Sweden) with the following settings: sampling rate of 384000, FFT size of 1024, FFT overlap of 0, 16 bits sample, and the Hanning window.

Results

At the PSU sites, a total of 9,744 5-sec recording windows were retrieved (PSU1: 4,704 and PSU2: 5,040, respectively); at PSU1, 364 (7.7 %) of these windows contained bat signals (dry season: 321; wet season: 43); at PSU2, bat signals occurred in 1,280 (25.4 %) of the windows examine (dry season: 572; wet season: 708). At the Bala site, 4,692 recording windows were retrieved in the wet season; 342 (7.3 %) of those time windows had bat signals. Thus, the highest numbers of bat signals were detected at PSU2 during the wet season.

Signals from ten species of bats were recognized (Figure 2 and Table 1), including three unidentified species: four CF (constant frequency) species - *Rhinilopus luctus* (Rl), *R. trifoliatus* (Rt), *R. acuminatus* (Ra), and *R. refulgens* (Rr), and six FM (frequency modulation) species - *Taphozous melanopogan* (Tm), Sonotype 1 (S1), *Scotophilus kuhlii* (Sk), Sonotype 2 (S2), *Myotis horsfieldii* (Mh), and *Kerivoula* sp. (K1). Sonotype 1 likely belonged to an unknown species of *Myotis* and Sonotype 2 to *Pipistrellus*.

The potential for interspecific interactions could be inferred from the time windows with multiple bat occurrences despite the low number of such time windows: only 81 (4.5 %) out of a total of 1,816 time windows had multiple bat calls. Among those 81 time windows, 47 time windows contained calls of *Myotis horsfeildii* co-occurring with six other species and 40 time windows contained calls of *Scotophilus kuhlii* co-occurring with five other species. Given that these two species were the second and third most common species in our study, these results

Table 1. Measurements for bat calls. fmaxe: frequency of maximal energy; sf: start frequency; tf: terminal frequency; d: call duration.

Species	*n*	(*fmaxe*: kHz)	(*sf*: kHz)	(*tf*: kHz)	(*d*: ms)
Rhinolophus luctus	4	41.3 ± 0.2 (41.1-41.5)	37.1 ± 0.9 (36.4-38.2)	38.8 ± 0.6 (38.4-39.7)	59.3 ± 2.7 (56.9-63)
Rhinolophus trifoliatus	19	52.7 ± 0.8 (51.3-53.5)	49.1 ± 3.3 (43-53.1)	47.9 ± 4.3 (41.4-52.6)	46.6 ± 6.4 (38-66)
Rhinolophus acuminatus	20	89.8 ± 2.7 (83.4-92.9)	77.9 ± 7.6 (68-92.1)	78.8 ± 7.9 (65.4-92.1)	51.7 ± 8.7 (35-67.5)
Rhinolophus refulgens	5	95.3 ± 0.7 (94.5-96.2)	76.6 ± 3.0 (72.6-80.3)	81.2 ± 10.8 (71.8-93.3)	50.2 ± 12.6 (29-61)
Taphozous melanopogan	3	28.6 ± 1.1 (27.7-29.8)	38.7 ± 4.8 (35.1-44.1)	24.5 ± 3.0 (21.5-27.5)	27.7 ± 36.3 (1-69)
Scotophilus kuhlii	16	40.8 ± 2.0 (35.9-43.1)	51.7 ± 7.0 (44-69.2)	38.7 ± 2.6 (34.7-42.6)	11.3 ± 4.0 (5.1-17)
Sonotype 1	1	40.7	56.1	30.1	6.9
Mytois horsfieldii	20	63.0 ± 6.4 (52.6-69.9)	97.6 ± 8.5 (80.7-107.7)	38.4 ± 3.4 (32.5-47.4)	5.0 ± 0.7 (3.8-6.8)
Sonotype 2	17	54.3 ± 3.9 (48.9-60.1)	75.7 ± 11.8 (58.4-92.1)	50.6 ± 2.8 (47.5-54.9)	4.7 ± 1.0 (2.3-6.9)
Kerivoula sp.1	11	127.2 ± 8.0 (105.5-133.1)	163.7 ± 2.0 (160-166.7)	93.0 ± 9.7 (80.8-103.7)	2.7 ± 0.4 (1.9-3.1)

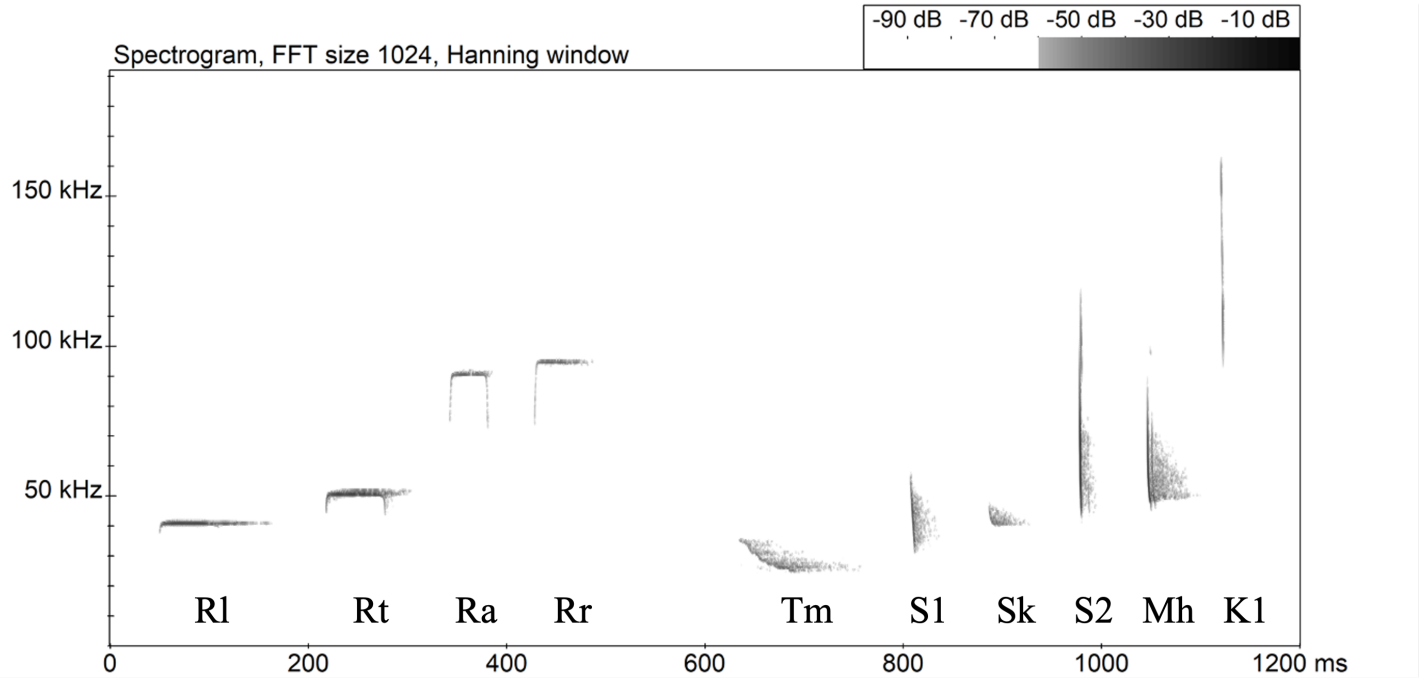

Figure 2. Spectrograms of representative calls of 10 bat species recorded at PSU and Bala. Four CF species: *Rhinilopus luctus* (Rl), *R. trifoliatus* (Rt), *R. acuminatus* (Ra), and *R. refulgens* (Rr). Six FM species: *Taphozous melanopogan* (Tm), *Sonotype* 1 (S1), *Scotophilus kuhlii* (Sk), *Sonotype* 2 (S2), *Myotis horsfieldii* (Mh), and *Kerivoula* sp. (K1).

are not surprising. In contrast, however, the most common species *Rhinolophus trifoliatus*, co-occurred with two other species in just 20 time windows. This, in part, may attest to the distinct foraging strategy of *Rhinolophus trifoliatus* using CF echolocation, which reduced the probability of their calls being recorded with other species in the same time window. Further testing will be needed to understand the spatial proximity associated with these acoustic co-occurrences.

Eight species and six species were recorded at PSU and Bala, respectively (Table 2). However, taking passing counts of 30 as a cutoff, only three species (*R. trifoliatus*, *M. horsfieldii*, and *S. kuhlii*) at PSU and two species (Sonotype 1 and *M. horsfieldii*) at Bala were considered common. Four species of *Rhinolophus* were detected in PSU but none in Bala, whereas *Kerivoula*, despite small passing counts, was detected only in Bala. Only one species (*M. horsfieldii*) was common at both PSU and Bala. Finally, hunting buzz calls were mostly detected at Bala (Table 2).

At PSU sites, seasonal variation in the calls recorded was only noted at PSU1, with more passing counts in the dry season than in the wet season (Table 2), primarily for *S. kuhlii* and *M. horsfieldii* (ten times or greater difference).

Diel activity patterns, as reflected by passing counts of four common species (*R. trifoliatus*, *S. kuhlii*, *M. horsfieldi*i, and Sonotype 2), were also examined (Figure 3). All except one species (*R. trifoliatus*) began to emerge at 17:00 hr, with *R. trifoliatus* emerging one hour later at 18:00 hr. At 06:00 hr, no passings were detected for Sonotype 2 while the other three species were still active at 07:00 hr. *R. trifoliatus* showed the most marked two-peak activity pattern, with a first peak at 21:00 hr and a second at 02:00 hr. More modest two-peak activity patterns were seen in the other three species, with varying peak times (Figure 3).

Discussion

Our trial of deploying automated bat recorders in forests in Southern Thailand was proven efficient in detecting diversity and activity patterns in a short period of recording time. Within one week, we documented 10 species of bats from their acoustic signals, including three unidentified species. Due to the continuous nature of the recordings, we were given a glimpse into diel activity patterns of these species as well, which would have been much more difficult to obtain without substantial effort to capture the bats. Finally, the close co-occurrence of different bat species could be inferred by examining overlapping of acoustic signals of multiple bat species within recording windows.

One drawback in attempting to identify bat species by their acoustic signals in this region is that only a few studies on bat echolocation are available for comparison despite the high level of bat diversity. Even though our identification for bat species based on acoustic signals relied largely upon a residential bat specialist (Pipat Soisok) who is experienced with local bat fauna and their call signals, we obtained some acoustic signals for which positive species identification could not be made. In those cases, species assignments were treated as tentative awaiting further clarification. Furthermore, knowledge on foraging behavior/ecology for some species has cast doubt on species identification. For instance, *Scotophilus kuhlii* (Zhu *et al.* 2012) and *Myotis horsfieldii* (Haslauer 2019) are known to be open-space foragers and yet their calls were common in our recordings despite the fact that our recorders were installed in lower levels

within forests. Conversely, the ability to detect behaviors not previously reported could turn out to be an under-appreciated merit of studying bats with automated recorders. Our current understanding of *Scotophilus kuhlii* is that this species is often associated with humans and congregates in roosting sites on cultivated palm trees (Zhu *et al.* 2012). At the Bala site, which is deep in a forested area, native palm trees are common and could be the original roosting sites for this species prior to human arrivals.

Three different habitat types were chosen for this study: PSU1, which includes a recently abandoned rubber plantation, PSU2, which is composed of less disturbed forest adjacent to a rubber plantation, and Bala, which is considered a natural forest. Our preliminary results indicate that once rubber plantations are set aside and ecological succession is allowed to proceed, bat species richness approaches that observed in natural forests. However, the composition of such assemblages (Table 2) differs in these two habitats, likely because structures of the forest types remain distinct and thus ecological niches within these habitat types still differ.

Due to its unique geographical position, Peninsular Thailand is a biodiversity hotspot, where Sundaland, Indochinese and Indo-Burmese faunal components converge and interchange (Myers *et al.* 2010). As a result, numerous national parks (39, including 4 proposed), forest parks (8), non-hunting areas (18), and wildlife sanctuaries (15) have been established in this region. Yet, land use in Southern Thailand is still shifting. Automated recordings have the potential not only to uncover aspects of bat ecology that were rarely known in the past, but also to monitor bat communities across these ever-changing landscapes.

Finally, diel activity patterns extracted from our data are interesting and important in revealing temporal niche separation by sympatric bat species. One example is *R. trifoliatus* and *M. horsfieldii,* which are sympatric at the PSU site (Table 2). While activity of *R. trifoliatus* reached peaks at 21:00 and 2:00 hr, peak times of *M. horsfieldii* were at 22:00 and 1:00 hr (Figure 3). Both of these species are known to forage in open space so temporal niche segregation could facilitate their co-occurrence. Because bats are the most mobile terrestrial mammals and are sensitive to subtle environmental changes, collecting acoustic signals at various structural positions within a forest to reveal diel activity patterns would provide a valuable means to understand their ecological roles and to monitor environmental changes.

In conclusion, our trials involving automated bat recorders are useful in documenting bat diversity, activity patterns of bats, and the potential for interspecific interactions. Nonetheless, studies of the acoustics of live bats that can be positively identified, preferably from a larger geographical realm, will increase the accuracy of assigning species identification to acoustic signals. Moreover, it is crucial that recorders be set in a wide variety of habitat structures and habitat types. For example, it will be important to position the recorders in a way that captures the vertical stratification within a forest as well as distinct topological features, such as in cleared open passageways or forest streams. Long-term collection and curation of bat calls using automated recorders will prove to be fruitful in expanding our understanding of the biodiversity of this region and the conservation of these changing landscapes.

Table 2. Passing counts of bats as detected by acoustic signals in the 5-second time windows.

Location-season/Species	PSU1_dry	PSU2_dry	PSU1_wet	PSU2_wet	BALA_wet	Sum
Rhinolophus luctus	12	13	0	26	2	53
Rhinolophus trifoliatus	1	344	19	553	0	917
Rhinolophus acuminatus	22	2	0	3	0	27
Rhinolophus refulgens	4	0	1	0	0	5
Taphozous melanopogan	5	1	0	3	0	9
Scotophilus kuhlii	177	61	11	57	29	335
Sonotype 1	0	0	0	0	1	1
Mytois horsfieldii	100	148	10	59	103	420
Sonotype 2	0	3	2	7	196	208
Kerivoula sp.1	0	0	0	0	11	11
Sum	321	572	43	708	342	1986
Feeding buzz	0	1	0	0	50	51
Total species	7	7	5	7	6	
		8			6	

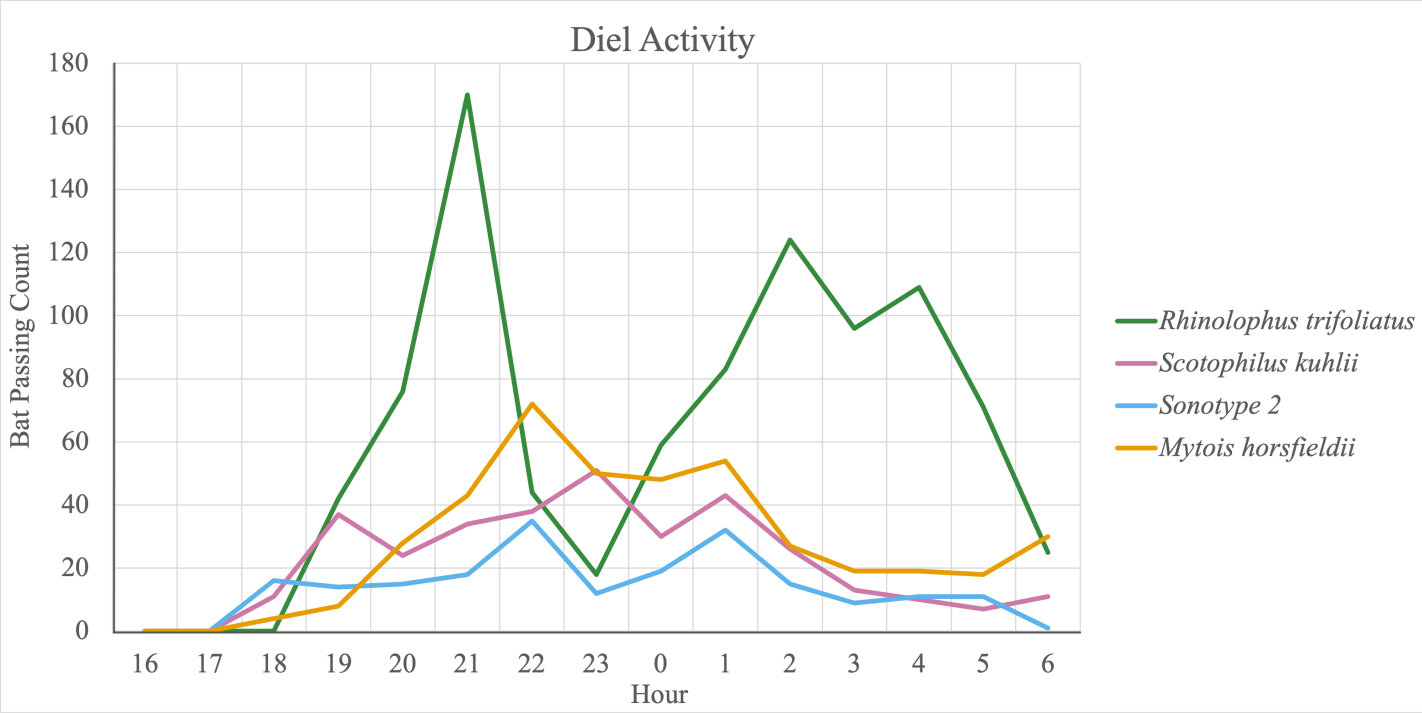

Figure 3. Diel activity patterns of four common species of bats in the southern region of Peninsular Thailand.

Acknowledgments

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Epilogue. In Feb 1986, I (Alex Yu) received a letter (done with a typewriter sent by snail mail) from a professor named James Patton, saying that he would accept me as a doctoral student and looked forward to working with me for several years. The English word "several" was just as vague as the city of Berkeley to me at the time. It turns out to be a precious relationship that has lasted for decades. Both Jim and Carol have become the backbone of this relationship. My wife Yulan (we got married in 1987) and later my daughter Grace (born in 2001) joined, too. The education and affection bestowed by Jim and Carol upon us are incredibly profound. We thank them with this little paper that was de facto started by that one-page hand-typed letter that reached me in 1986.

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Plate 1: Forested slope adjacent to the campus of Prince Songkla University in Peninsular Thailand. This site is an abandoned rubber plantation.

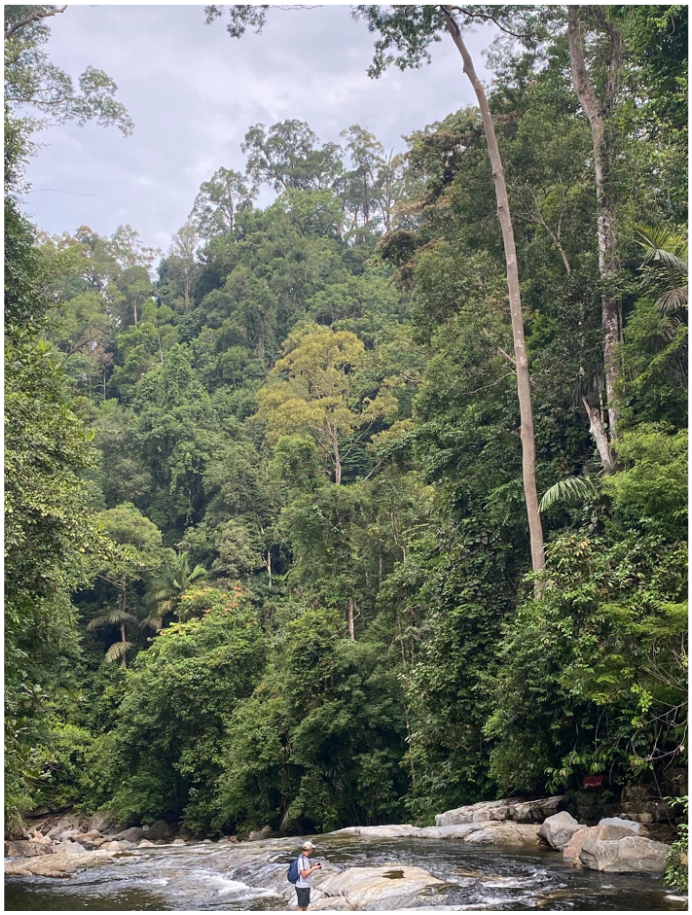

Plate 2. Natural tropical rainforest in Hala-Bala Wildlife Sanctuary. Dipterocarps are dominant tree species here.

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Mammals of Fish Springs National Wildlife Refuge, Utah: assessing diversity, and effects of long-term wetland management on native and introduced species

Eric A. Rickart[1]*, Rebecca J. Rowe[2], Alexandra C. Coconis[3], and Shannen L. Robson[4]

[1]Natural History Museum of Utah, University of Utah, 301 Wakara Way. Salt Lake City, UT 84108, U.S.A. Email: rickart@umnh.utah.edu (EAR).

[2]Department of Natural Resources and the Environment, University of New Hampshire, 56 College Road. Durham, NH 03824, U.S.A. Email: rebecca.rowe@unh.edu (RJR).

[3]Department of Natural Resources and Environmental Science, University of Nevada, 1664 North Virginia Street. Reno, NV 89557, U.S.A. Email: acoconis@unr.edu (ACC).

[4]Natural History Museum of Los Angeles County, 900 Exposition Blvd. Los Angeles, CA 90007, U.S.A. Email: srobson@nhm.org (SLR).
*Corresponding author: Eric A. Rickart http://orchid.org/0000-0002-6274-0501.

Fish Springs National Wildlife Refuge, one of the most isolated wildlife refuges in the lower 48 US states, was established with the singular purpose of transforming a natural desert wetland into managed habitat for migratory waterfowl. The longitudinal effects of wildlife management on non-target species are rarely examined. In this study, we used specimen-based evidence from historical and modern surveys to 1) compile a list of the mammal fauna of Fish Springs NWR, 2) identify other regional species from within the eastern Great Basin that may occur on the refuge but have yet to be detected, and 3) assess how 60 years of intensive waterfowl management may have affected mammal diversity and abundance. Results document 33 species of mammals currently (or formerly) present at Fish Springs. It is likely that several additional species, primarily small mammals (shrews, bats, and rodents), are present on the refuge. One species thought to have been introduced during the 20th century, was in fact present prehistorically, and one non-native species that was introduced appears to have suffered a recent population crash. Changes in species' abundance were evident as a result of habitat modifications and management practices, but otherwise, there is no evidence of major impact on native mammals.

El Refugio Nacional de Vida Silvestre Fish Springs, uno de los refugios más aislados en los 48 estados inferiores de EE. UU., se estableció con el singular propósito de transformar un humedal desértico natural en un hábitat gestionado para aves acuáticas migratorias. Rara vez se examinan los efectos longitudinales del manejo de la vida silvestre en especies no objetivo. En este estudio, utilizamos evidencia basada en especímenes de estudios históricos y modernos para: 1) compilar una lista de la fauna de mamíferos de Fish Springs NWR, 2) identificar otras especies regionales dentro del Great Basin oriental que pueden existir en el refugio, pero que aún no han sido registrados, y 3) evaluar cómo 60 años de manejo intensivo de aves acuáticas pueden haber afectado la diversidad y abundancia de los mamíferos. Los resultados registran 33 especies de mamíferos presentes actualmente (o anteriormente) en Fish Springs. Es probable que adicionalmente varias especies, principalmente pequeños mamíferos (musarañas, murciélagos y roedores), estén presentes en el refugio. Una especie, que se cree fue ampliamente introducida durante el siglo XX, pero con registros prehistórico, y otra no nativa introducida parece haber tenido una disminución demográfica reciente. Los cambios en la abundancia de especies fueron evidentes como resultado de modificaciones del hábitat y prácticas de gestión, pero por lo demás, no hay evidencia de un impacto importante en los mamíferos nativos.

Keywords: Biodiversity, desert wetland, faunal survey, Great Basin, habitat disturbance, land use, natural history, resource management.

Introduction

A primary function of the U.S. Fish and Wildlife Service (USFWS) has been the development and maintenance of refuges that provide protected habitat for the conservation of fish and wildlife. Historically, individual refuges were established for a variety of specific management purposes but were not guided by a uniform mission statement (Schroeder 2008). The National Wildlife Refuge System Administration Act of 1966 (16 U.S. Code § 668dd) formally established the USFWS refuge system. The mission of the wildlife refuge system was clarified under the National Wildlife Refuge System Improvement Act of 1997 (Public Law 105-57. 111 Statute 1252) as the "conservation, management, and where appropriate, restoration of the fish, wildlife, and plant resources and their habitats". Under this comprehensive mandate, the Fish and Wildlife Service is concerned with assessing the effects of refuge management practices on non-target wildlife.

Fish Springs National Wildlife Refuge (Figure 1) was established in 1959 specifically to create habitat for migratory waterfowl in an area of natural desert wetland in western Utah (U.S. Fish and Wildlife Service 2004). Between May 1961 and October 1964, the natural discharge from a series of existing geothermal springs was altered with the construction of dikes and canals resulting in a network of engineered wetland habitat managed through regulated flooding, prescribed burns, mowing, and herbicide treatments (U.S. Fish and Wildlife Service 2004).

Habitat manipulation for conservation and management purposes can have immediate impacts on the abundance and occurrence of small mammals (Zou *et al.* 1989; Mitchell *et al.* 1995; Fitzgerald *et al.* 2001). Because they are sensitive to changes in vegetation and microclimate and have short generation times and high reproductive potential, small mammals can be excellent indicators of landscape change and ecosystem health. However, species responses unfold over time through stages of development among management treatments that differentially shape the composition and structure of vegetation. Thus, the impacts reported in previous studies may only reflect short-term changes rather than long-term responses of small mammal populations and communities (Swihart and Slade 1990; Brady and Slade 2001; Fernández *et al.* 2021; Fuentes *et al.* 2020).

As an isolated wetland within the arid eastern Great Basin, Fish Springs provides important ecological services in supporting a range of biodiversity. As an area with a long and successful history of exclusive management directed toward one particular faunal group (birds), Fish Springs also provides an opportunity to study long-term effects of this management approach on other faunal groups. In this study, we use data from museum records and modern field surveys to examine how six decades of directed waterfowl management at Fish Springs has affected its mammal fauna.

Materials and methods

Study area and history. Fish Springs National Wildlife Refuge (hereafter "Fish Springs" or "refuge") is located on the northeastern flank of the Fish Springs Range at the southern margin of the Great Salt Lake Desert in western Juab Co., Utah (Figure 1). The refuge encompasses 7,280 ha with elevations ranging from 1,305 m along the eastern boundary on the Fish Springs Flat to 1,450 m in the northeastern-most portion of the Fish Springs Range. A series of geothermal springs located along the eastern base of the Fish Spring Range have a combined annual discharge of approximately 32 million m^3 (Gates and Kruer 1981). These springs support a marsh wetland with surrounding vegetated sand dunes on the west and south and desert playa to the north and east.

Fish Springs is located within the Bonneville Basin, which defines the eastern-most portion of the Great Basin. The region was under water from about 26 Ka when Lake Bonneville began to fill (Grayson 2011) to about 11.4 Ka at the end of the Gilbert episode (Oviatt 2014) with the subsequent development of wetland habitat. Archaeological

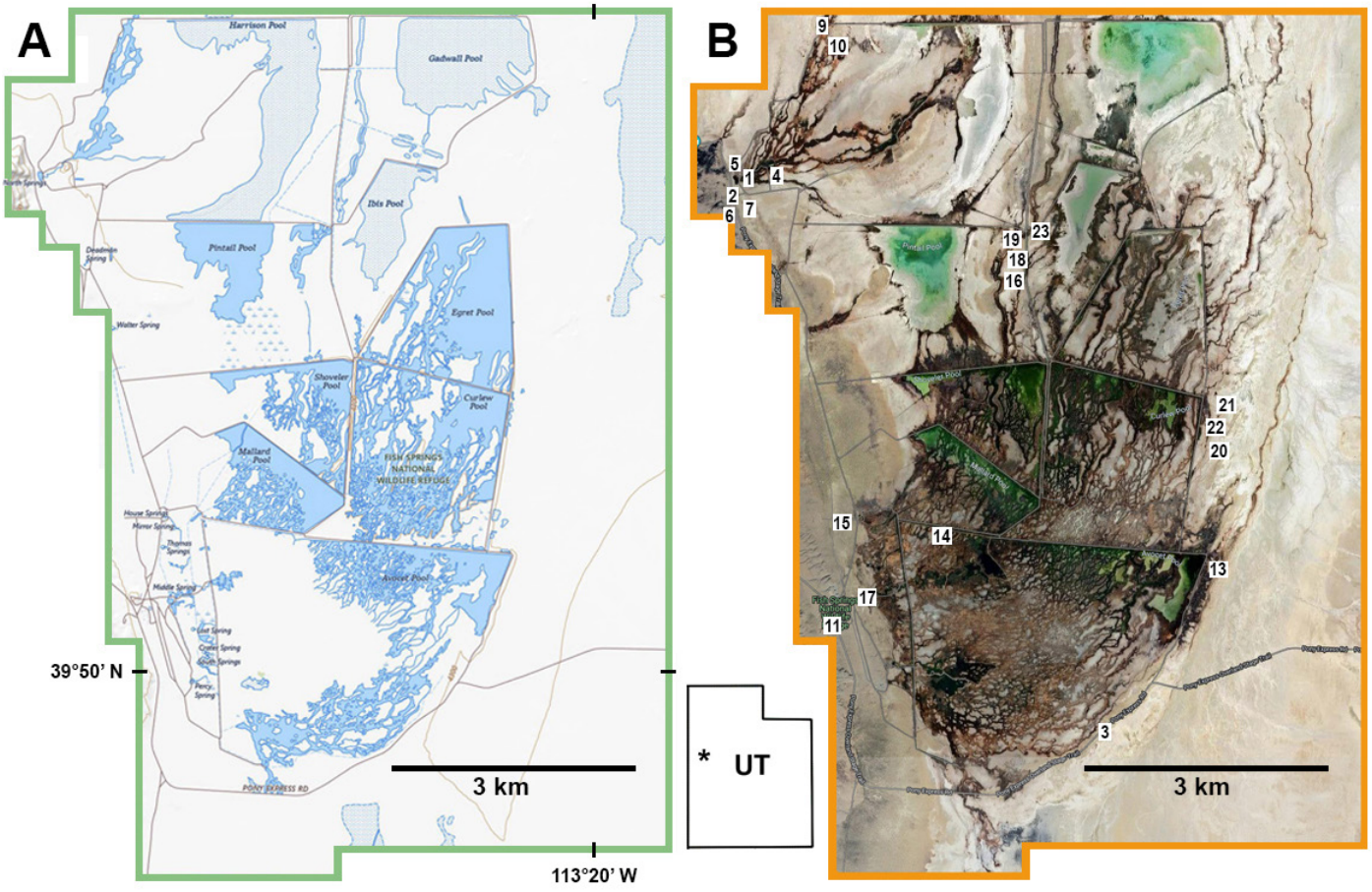

Figure 1. Fish Springs National Wildlife Refuge in western Utah (inset map). A) U.S. Geological Survey topographic map showing spring discharge and areas of open water in managed wetland (dark blue) and areas of managed meadow and seasonal playas (gray). B) Satellite image from Berkeley Mapper (Arctos) with trapping locations numbered as in Table 1.

surveys of two Fish Springs cave sites indicate prehistoric occupancy by late Archaic peoples between 2.5 and 4.0 Ka based on radiocarbon dating of charcoal and associated archaic projectile points (Madsen 1982). Projectile points recovered from upper stratigraphic layers revealed occupation by post-Archaic people after 1800 BP.

Fish Springs is within the ancestral lands of the Goshute (*Newe*) people who were hunter-gatherers in this region until the early 1900s (USFWS 2004). The earliest documented occupation by Euro-Americans was in 1859 to 60 with the establishment of Pony Express and Overland Stage stations. In the 1890s, John J. Thomas established a cattle and horse ranch at Fish Springs, and in 1913, the Lincoln Highway was built to accommodate automobile travel (Joy 1916). The Fish Springs Livestock and Fur company was established in 1925 (USFWS 2004). These human activities involved direct exploitation of large game and other resources and direct habitat disturbance. In 1959, Fish Springs was purchased by the US Fish and Wildlife Service with subsequent major modifications to the marsh ecosystem as described above.

Habitats. The refuge includes five principal terrestrial habitats (Table 1) distinguished by vegetation and edaphic features (Robson and Rickart 2004; USFWS 2004; also see Bolen 1964; Emrick and Hill 1998).

Spring edge wetland. Areas surrounding the margins of natural springs with shallow water or saturated soils support vegetation dominated by common reed (*Phragmites australis*), cattails (*Typha domingensis, T. latifolia*), bulrushes (*Scirpus acutus, S. americanus,* and *S. maritimus*), Baltic rush (*Juncus arcticus*), saltgrass (*Distichlis stricta*), alkali sacaton (*Sporobollus airoides*), sea milkwort (*Glaux maritima*), alkali weed (*Cressa truxillensis*), and boraxweed (*Nitrophila occidentalis*).

Marsh meadow. Meadows adjacent to springs and water diversion are managed through periodic flooding, prescribed burns, and mowing. Vegetation in these areas is dominated by common reed, saltgrass, Baltic rush, and alkali sacaton.

Salt desert shrubland. Playa bottoms with seasonally saturated saline soils principally in the eastern and southern portions of the refuge support plant communities dominated by greasewood (*Sarcobatus vermiculatus*), iodine bush (*Allenrolfea occidentalis*), saltlover (*Halogeton glomeratus*), shadscale (*Atriplex tridentata*), greenmolly (*Bassia americana*), seepweed (*Suaeda moquinii*), pickleweed (*Sarcocornia utahensis*), and saltgrass.

Sand dune. Several groups of vegetated sand dunes are located along the refuge boundaries within areas of shrubland. Dominant plants included greasewood, shadscale, iodine bush, pickleweed, flatcrown buckwheat (*Eriogonum deflexum*), and saltgrass.

Mixed arid shrubland. On the western margin of the refuge, rocky slopes along the eastern base of the Fish Spring Mountains support vegetation dominated by Mormon tea (*Ephedra nevadensis*), fourwing saltbush (*Atriplex canescens*), shadscale, chamisa (*Ericameria nauseosa*), horsebrush (*Tetrodymia spinosa* and *T.* sp.), broom snakeweed (*Gutierrezia sarothrae*), Indian ricegrass (*Achnatherum hymenoides*), and cheatgrass (*Bromus tectorum*).

History of mammal collecting at Fish Springs. The earliest voucher specimens from Fish Springs (a series of muskrats) were obtained in 1934 by Vasco M. Tanner from Brigham Young University (BYU specimens). In June 1940 Stephen D. Durrant and Henry W. Setzer (University of Utah) trapped at localities in shrubland, grassland and marsh habitats where they documented eight rodent species (Natural History Museum of Utah [UMNH] specimens). In 1959, Guy Musser obtained specimens of fur-bearing mammals from a resident trapper at Fish Springs, documenting the occurrence of seven carnivoran species (UMNH specimens). Several other individuals including C. Lynn Hayward (BYU), Harold J. Egoscue and John B. Bushman (Dugway Proving Ground) added small mammal specimen records during the 1950's and 60's (BYU and UMNH specimens). In July 1992, Dana J. Shurtleff (BYU) collected mammals from the refuge and surrounding habitat, documenting 17 mammal species including shrews, bats, rodents and lagomorphs (BYU specimens). From September 2002 to October 2003, Shannen Robson (UMNH) trapped small mammals (rodents and shrews) at 21 locations in all major habitats at Fish Springs, documenting habitat associations and seasonal abundance of 12 species (Robson and Rickart 2004). From 2012 to 2018, Robson and Rickart continued to periodically survey small mammals at Fish Springs, documenting two additional species.

Recent Field Methods (2002–2018). Traplines consisting of Sherman live traps were baited with oatmeal and birdseed, and Museum Special and Victor Rat traps were baited with a mixture of oatmeal and peanut butter. We opened traps for 1 to 3 days and nights, closing them only during the midday to avoid extreme heat, and checked them at dawn and dusk. Additionally, we collected pocket gophers using Macabee traps. Bats were captured in mist nets set along the margin of North Spring for one night only. Some live-trapped individuals of the most common species were released at the point of capture after identification. All others were preserved as voucher specimens and deposited at the Natural History Museum of Utah, with records available through the Arctos Database Consortium (Cicero *et al.* 2024; https://arctos.database.museum/; accessed 17-11-2024).

Field surveys were conducted under permits from the US Fish and Wildlife Service (USFWS 65540–02004; 65440–201213) and the Utah Division of Wildlife Resources (UDWR 1COLL14). Field methods followed guidelines established by the American Society of Mammalogists (Gannon *et al.* 2007), and were approved by the University of Utah Institutional Animal Care and Use Committee (IACUC protocols 02-08003, 12-01001 and 15-02001).

Specimen Records. We compiled museum specimen records through Arctos and from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org; accessed

Table 1. Small mammal (rodent and shrew) trapping data at sites in five major habitat types at Fish Springs National Wildlife Refuge, Juab County, Utah, USA. Data are from surveys conducted in 2002 and 2003 (UMNH records).

Site number	Habitat type	Decimal latitude	Decimal longitude	Elev (m)		Total trap nights	Specimens collected	Animals released	Trap success (%)
1	spring edge	39.88758°	113.41237°	4,281		57	8	0	14.04
10	"	39.90414°	113.40073°	4,281		62	14	0	22.58
16	"	39.87831°	113.37464°	4,280		30	17	1	60.00
					habitat totals	149	39	1	26.85
4	marsh meadow	39.88725°	113.40827°	4,305		112	1	0	0.89
5	"	39.88758°	113.41237°	4,281		90	6	1	7.78
14	"	39.84967°	113.38392°	4,301		30	3	0	10.00
18	"	39.87877°	113.37532°	4,276		15	6	0	40.00
22	"	39.85872°	113.34547°	4,296		10	1	0	10.00
					habitat totals	257	17	1	7.39
7	salt shrubland	39.88449°	113.41314°	4,250		89	6	0	6.74
19	"	39.87907°	113.37540°	4,278		30	1	3	13.33
23	"	39.88207°	113.36964°	4,302		60	2	1	5.00
					habitat totals	179	9	4	7.26
3	sand dune	39.82750°	113.35964°	4,311		80	9	0	11.25
9	"	39.90428°	113.40133°	4,314		100	9	15	24.00
13	"	39.84546°	133.34512°	4,306		60	3	0	5.00
17	"	39.84134°	133.39370°	4,319		30	3	8	36.67
20	"	39.85682°	133.34330°	4,295		38	3	4	18.42
21	"	39.85969°	133.34217°	4,293		74	4	3	9.46
					habitat totals	382	31	30	15.97
2	mixed shrubland	39.88458°	113.41361°	4,300		20	3	0	15.00
6	"	39.88389°	113.41447°	4,370		91	11	0	12.09
11	"	39.83839°	113.39954°	4,346		80	8	18	32.50
15	"	39.84927°	113.39965°	4,350		40	8	4	30.00
					habitat totals	231	30	22	22.51
					grand totals	1,198	126	58	15.36

14-09-2024) including all records for specimens collected from the Fish Springs National Wildlife Refuge (or simply "Fish Springs" for those collected before the establishment of the refuge).

To document the regional diversity of mammals within the eastern Great Basin, including species that have been reported to occur on the refuge or those that may occur there but are not represented by voucher specimens, we compiled regional museum specimen records from localities within a 200 km radius of Fish Springs in Juab, Millard and Tooele counties in Utah, and Elko and White Pine counties in Nevada (Figure 2). We selected single voucher specimens from the nearest localities to Fish Springs in each of the five counties, resulting in from 1 to 15 specimen records per species; sufficient to assess regional diversity of species, but not reflecting their broader distribution or relative abundance. For a few species that have been reported from the refuge on the basis of sightings, use of bat detectors, or capture and subsequent release but are not represented by regional specimens, we report the nearest specimen records beyond the defined region. For two species that lack vouchered records, we cite recent iNaturalist sightings from Fish Springs.

We retrieved specimen records from the following institutions (with collection acronyms): Brigham Young University Life Science Museum (BYU), California State University, Long Beach (CSULB), Denver Museum of Nature and Science (DMNS), Cal Poly Humboldt Vertebrate Museum (HSUVM), Museum of Natural Science, Louisiana State University (LSUMZ), Museum of Southwestern Biology, University of New Mexico (MSB), Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), Natural History Museum of Utah, University of Utah (UMNH), University of Montana Zoological Museum (UMZM), University of Nevada, Reno (UNR), Burke Museum of Natural History and Culture, University of Washington (UWBM), University of Wyoming Museum of Vertebrates (UWYMV). We verified the identification of all legacy specimens from UMNH. Duke S. Rogers verified identifications of legacy Fish Springs specimens from BYU. All specimen records are listed in Appendix 1.

Archaeological records. In 1979, test excavations were made at Barn Owl and Crab Caves located at the northern end of the Fish Springs Range within the northwestern margin of the refuge (Madsen 1982). Prehistoric faunal remains recovered by the Division of Utah State History Antiquities Division are housed in UMNH Anthropology collections.

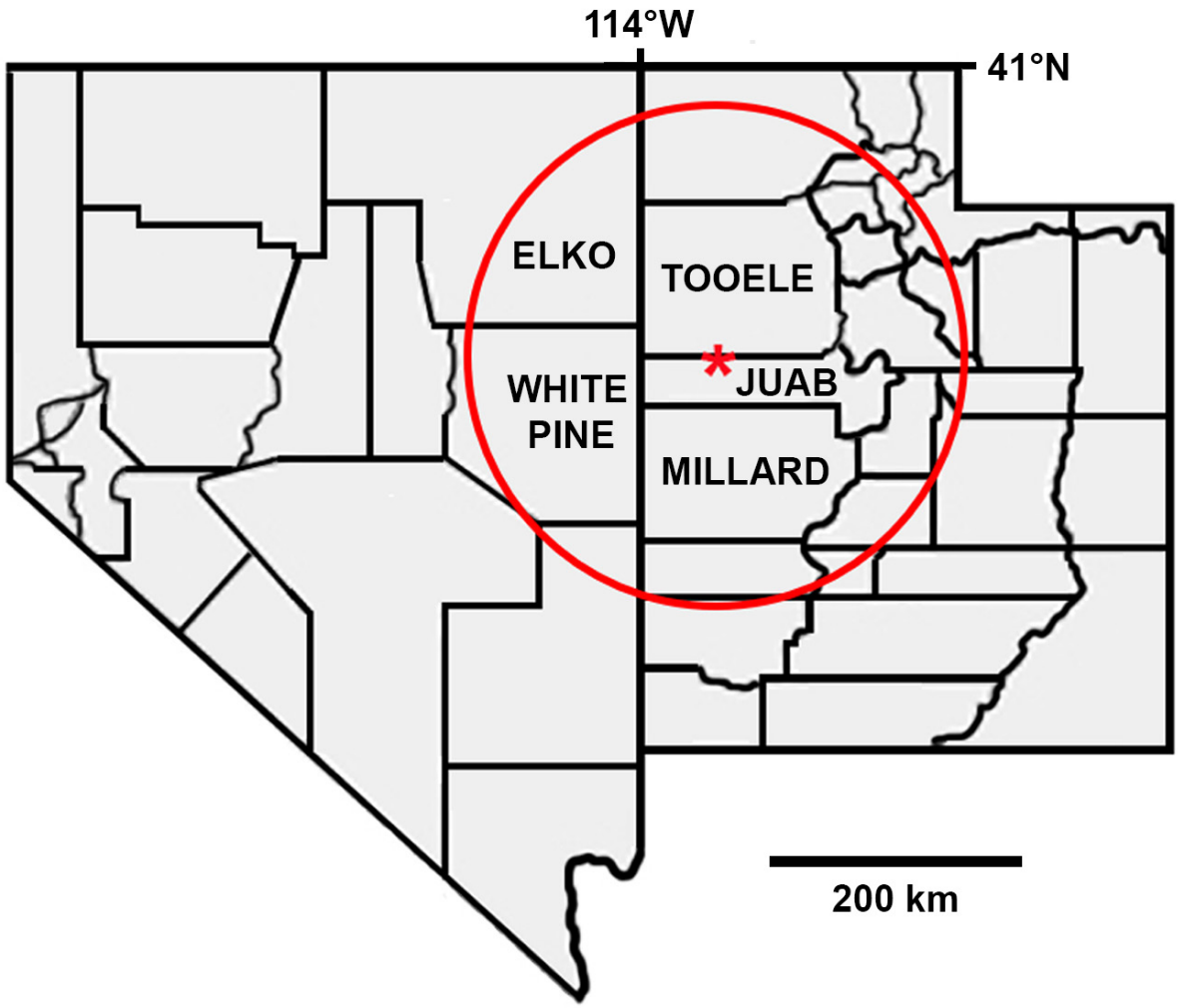

Figure 2. Map of Utah and Nevada showing location of Fish Springs (red star) in Juab County, Utah, and adjacent counties in both states. Red circle denotes the area within a 200 km radius of Fish Springs from which regional specimen records were retrieved.

They include identified skeletal elements of 10 mammal species (*Canis latrans*, *Antilocapra americana*, *Odocoileus hemionus*, *Ovis canadensis*, *Lepus californicus*, *Sylvilagus audubonii*, *Thomomys bottae*, *Neotoma* sp. *Microtus* sp., and *Ondatra zibethicus*) dated between *ca*. 4000 and 1800 BP (Madsen 1982).

Species Accounts

Eulipotyphla

Soricidae (5 species, 2 of which are documented from the refuge)

Notiosorex crawfordi (Baird, 1877)

Crawford's Gray Shrew

Fish Springs records. None.

Regional records. 4: Utah, Tooele County, Dugway Proving Grounds, site 18 C (BYU), Granite Mountain, Site 15 A (BYU), Wig Mountain (BYU), Sapphire Mountain (BYU). This species occurs in arid and semiarid habitats, including desert shrub, grassland, alkaline marsh and mud flats (Carraway and Timm 2000) all of which are extensive on the refuge and similar to habitat at the nearby regional localities in Tooele County. Its occurrence at Fish Springs is very likely.

Sorex merriami Dobson, 1890

Merriam's Shrew

Fish Springs records. None.

Regional records. 5: Utah, Juab County, 10 mi. S Eureka (MSB); Tooele County, 6 mi. S Vernon (MSB); Nevada, Elko County, Adobe Hills (UMNH). White Pine County, 2 mi. S Baker (MVZ), Strawberry Canyon (UMNH). A widespread but uncommon species, often associated with sagebrush (*Artemisia*; Armstrong and Jones 1972). It may occur in the Fish Springs Range along the western margin of the refuge.

Sorex preblei Jackson, 1922

Preble's Shrew

Fish Springs records. 1: vicinity of North Spring (UMNH).

Regional records. 3: Utah, Tooele County, Horseshoe Spring (BYU); Timpie Springs (UMNH); Nevada, Elko County,

Ruby Valley USFS station (UMNH). This is an uncommon species found in a wide variety of habitats, including alkaline habitat dominated by halophytic plants (Tomasi and Hoffmann 1984; Cornely *et al.* 1992). The single specimen from Fish Springs was trapped in salt desert shrubland habitat dominated by saltgrass (*Distichlis stricta*) pickle weed (*Allenrolfea occidentalis*), and swampfire (*Salicornia utahensis*). This shrew may be present throughout much of the refuge in habitat dominated by halophytic plants.

Sorex tenellus Merriam, 1895

Inyo Shrew

Fish Springs records. None.

Regional records. 4: Utah, Juab County, Granite Creek Canyon (UMNH); Nevada, Elko County, Lamoille Canyon (UMNH); White Pine County, Lehman Creek (UMNH), Bald Mountain (UMNH). This is a poorly known species, generally found in talus and other rocky habitat across a broad elevational range (Hoffmann and Owen 1980; Rickart *et al.* 2017). It may occur in suitable rocky habitat along the western margin of the refuge.

Sorex vagrans Baird, 1857

Vagrant Shrew

Fish Springs records. 46: (BYU and UMNH).

Regional records. 7: Utah, Juab County, 3.6 km E, 3.5 km S summit Ibapah Peak, Deep Creek Range (BYU), Granite Creek Canyon (UMNH); Tooele County, Timpie Springs (UMNH), 7 mi N Iosepa (UMNH); Nevada, Elko County, Ruby Lake (MVZ); White Pine County, Cleveland Ranch (MVZ), Snake Range, mouth of Pole Canyon (UMNH). At Fish Springs, vagrant shrews are relatively abundant, but restricted to mesic habitats (Table 2).

Chiroptera

Molossidae (1 regional species not documented from the refuge)

Tadarida brasiliensis (I. Geoffroy, 1824)

Brazilian Free-tailed Bat

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 7: Utah, Juab County, 4 mi N Nephi (CSULB); Millard County, Holden (UMNH); Tooele County, Dugway (UMNH), 4 mi E Camelback Mountain (UMNH), Cedar Mountains (UMNH); Nevada, White Pine County, Baker (UMNH), Snake Range, Rose Guano Cave (UMNH). This species is very widespread and roosts in both caves and buildings (Wilkins 1989). Given the proximity of regional records from Tooele County, this species likely occurs on the refuge.

Vespertilionidae (13 species, 5 of which are documented from the refuge)

Antrozous pallidus (LeConte, 1856)

Pallid Bat

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 5: Utah, Juab County, Callao (UMNH); Millard County, Desert Experimental Range Station (UWYMV); Tooele County, Dugway (UMNH), 2 mi NE Camelback Mountain (UMNH); Nevada, White Pine County, Rose Guano Cave (UMNH). As a widespread species associated with arid habitats, rocky outcrops and water sources (Hermanson and O'Shay 1983), it likely occurs at Fish Springs.

Corynorhinus townsendii (Cooper, 1837)

Townsend's Big-eared Bat

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 9: Utah, Juab County, 12 mi W Fish Springs Wildlife Refuge (UMNH), Trout Creek Canyon (UMNH); Millard County, Gandy Mountain Cave (UMNH). Tooele County, Dugway Mountains (UMNH), Little Granite Mountain (UMNH), Gold Hill (UMNH); Nevada, Elko County, 1 mi N, 2 mi E Carlin (UNR); White Pine County, Schell Creek Range (UMNH), Cleveland Ranch (MVZ). This species is common throughout the region. It roosts in caves and mines (Kunz and Martin 1982) and is likely to forage and roost on or near the refuge.

Eptesicus fuscus (Palisot de Beauvois, 1796)

Big Brown Bat

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 7: Utah, Juab County, Callao (UMNH), Cherry Creek (LSUMZ); Millard County, Oak City (UMNH); Tooele County, 2 mi E Ibapah (UMNH), 0.5 mi W Johnsons Pass (UMNH); Nevada, Elko County, Ruby Valley USFS Station (UMNH); White Pine County, Baker (UMNH). This species is regionally common and frequently roosts in buildings but also uses mines and caves (Kurta and Baker 1990); it likely occurs on the refuge.

Lasionycteris noctivagans (LeConte, 1831)

Silver-haired Bat

Fish Springs records. 1: (UMNH).

Regional records. 4: Utah, Juab County, 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU), Callao (UMNH); Millard County, Oak Creek Reservoir (UMNH);

Table 2. Small mammal (rodent and insectivore) occurrence records across five major habitat types at Fish Springs National Wildlife Refuge, Juab County, Utah, USA. Data from surveys conducted from 2002 to 2018 (UMNH records).

Species	Spring edge wetland	Marsh meadow	Salt desert shrubland	Sand dune	Mixed arid shrubland	Totals	No. of occupied habitats
Ammospermophilus leucurus			3	2	11	16	3
Chaetodipus formosus					18	18	1
Dipodomys microps			3	24	8	35	3
Dipodomys ordii				4	1	5	2
Microtus montanus	34	4	1	1		40	4
Mus musculus	22	6		2	1	31	4
Neotoma lepida					13	13	1
Onychomys leucurus					1	1	1
Peromyscus crinitus					22	22	1
Peromyscus maniculatus	16	1	10	53	24	104	5
Reithrodontomys megalotis	28		6	30	2	66	4
Sorex preblei			1			1	1
Sorex vagrans	32	13				45	2
Total	132	24	24	116	101	397	

Tooele County, 2 mi NE Camelback Mountain (UMNH). This species is regionally common, and uses a wide variety of day roosts and hibernacula (Kunz 1982).

Lasiurus cinereus (Palisot de Beauvois, 1796)

Northern Hoary Bat

Fish Springs records. No specimen records; one recent iNaturalist record (2015; https://www.inaturalist.org/observations/2385480).

Regional records. 3: Utah. Juab County, 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU); Tooele County, 3 mi N Iosepa (UMNH), Dugway Proving Grounds (BYU). This species principally roosts in trees (Shump and Shump 1982), and its occurrence on the refuge may be restricted by the lack of suitable roosts.

Myotis californicus (Audubon and Bachman, 1842)

California Myotis

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 3: Utah, Millard County, Muddy Spring, House Range (MSB); Tooele County, 5 mi N Ibapah (UMNH); Nevada, Elko County, Spruce Mountain (HSUVM). Distribution maps exclude this species from portions of the Great Basin (Zeveloff 1988; Simpson 1993), however nearby regional records from Tooele and Millard counties suggest that it likely occurs at Fish Springs.

Myotis ciliolabrum (Merriam, 1886)

Western Small-footed Myotis

Fish Springs records. 5: (BYU and UMNH).

Regional records. 7: Utah, Millard County, 4 mi E Oak City (UMNH); Tooele County, 1 mi E Ibapah (UMNH), Parrish Ranch, 5 mi N Ibapah (UMNH), 5 mi N Camelback Mountain (UMNH), south end of Cedar Mountains (UMNH); Nevada, Elko County, Ruby Lake (MVZ); White Pine County, Mt. Moriah (MVZ). Based on the number of specimens, this is the most abundant bat at Fish Springs.

Myotis evotis (H. Allen, 1864)

Long-eared Myotis

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 6: Utah, Juab County, Cherry Creek (LSUMZ); Millard County, Oak Creek Canyon (UMNH); Tooele County, Harker Canyon (BYU), southwest base of Stansbury Mountains (UMNH); Nevada, Elko County, Spruce Mountain (HSUVM); White Pine County, 2 mi W Smith Creek Cave, Mt. Moriah (MVZ). A common regional species that occurs in a wide range of habitats but is most common in forest (Manning and Jones 1989). It likely occurs on the refuge.

Myotis lucifugus (LeConte, 1831)

Little Brown Myotis

Fish Springs records. 2: (UMNH).

Regional records. 5: Utah, Juab County, 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU), York (UMNH); Millard County, Pavant Range, Robins Valley (UMNH); Tooele County, Skull Valley (UMNH); Nevada, Elko County, Ruby Lake (MVZ). This is a common regional species.

Myotis thysanodes Miller, 1897

Fringed Myotis

Fish Springs records. None. Included in the refuge species list (USFWS 2004) but there are no vouchered records.

Regional records. None. There are no recorded specimen records for this species from within the Great Basin. The closest vouchered records are from Garfield (MSB), Utah (BYU), Washington (UMNH) and Wayne (MSB) counties in Utah, and Clark County, Nevada (CSULB and MSB). Although this species uses a wide variety of habitats including arid shrublands (O'Farrell and Studier 1980), the absence of regional specimen records suggests that it is unlikely to occur at Fish Springs.

Myotis volans (H. Allen, 1866)

Long-legged Myotis

Fish Springs records. 1: (BYU).

Regional records. 5: Utah, Juab County, Deep Creek Mountains, Basin Creek Pass (UMNH), Cherry Creek (LSUMV); Tooele County, Cedar Mountains (UMNH), Sheeprock Mountains (BYU); Nevada, Elko County, Spruce Mountain (HSUVM); White Pine County, 7 mi SE Mt Moriah (MVZ).

Myotis yumanensis (H. Allen, 1864)

Yuma Myotis

Fish Springs records. None. Included in the refuge species list (USFWS 2004) but there are no vouchered records.

Regional records. none. There are no recorded specimen records for this species from the eastern Great Basin, where they appear to be excluded in published range maps (Zeveloff 1988; Braun *et al.* 2015). The closest vouchered records in Utah are from Cache (BYU), Emery (BYU), Utah (BYU), Iron (UMNH) and Washington (UMNH) counties, plus additional records from Clark County, NV (MSB) and Owyhee County, ID (MVZ). This species is unlikely to occur at Fish Spring.

Parastrellus hesperus (H. Allen, 1864)

Canyon Bat

Fish Springs records. 4: (BYU and UMNH).

Regional records. 3: Utah, Tooele County, Staley Spring, Dugway Range (UMNH), 18 mi SW Orr's Ranch, Skull Valley (UMNH), 3 mi E Camelback Mountain (UMNH). There are relatively few regional records for this species. Fish Springs is the only vouchered locality in Juab County.

Carnivora

Felidae (2 species, 1 of which is documented from the refuge)

Lynx rufus (Schreber, 1777)

Bobcat

Fish Springs records. 8: (UMNH).

Regional records. 10: Utah, Juab County, Trout Creek (UMNH), 7 mi W Joy (UMNH); Millard County, 4 mi S Gandy (MVZ), 30 mi W Delta (BYU); Tooele County, Gold Hill (UMNH), Dugway Valley (UMNH), Simpson Mountains (UMNH); Nevada, Elko County, Spruce Mountain (MVZ); White Pine County, Snake Range (UMNH), Duck Creek (MSB). This species is likely common as a predator of small and medium-sized mammals.

Puma concolor (Linnaeus, 1771)

Mountain Lion

Fish Springs records. None.

Regional records. 7: Utah, Juab County, Deep Creek Mountains, Granite Creek Canyon (UMNH), mouth of Red Cedar Canyon (UMNH); Millard County, Eightmile Creek (UMNH); Nevada, Elko County, Cold Creek, Ruby Mountains (MVZ); White Pine County, Snake Range, Chokecherry Creek (MVZ), Great Basin National Park, near Baker Creek (UMNH), Big Wash (UMNH). Although mountain lions are regionally common, they are dependent upon presence of native ungulate prey (Currier 1983), and their occurrence at Fish Springs would likely be sporadic.

Canidae (4 species, 2 of which are documented from the refuge)

Canis latrans Say, 1823

Coyote

Fish Springs records. 2: (UMNH).

Regional records. 8: Utah, Juab County, Cane Springs (UMNH), 7 mi W Joy (UMNH), Trout Creek (UMNH); Millard County, 6 mi W Deseret (MVZ), Garrison (MVZ); Tooele County, Grassy Mountains (UMNH). Nevada, Elko County, 5 mi W Wendover (UMNH); White Pine County, Steptoe Creek (MVZ). Coyotes are commonly observed in and around the refuge (USFWS 2004). Madsen (1982) reported prehistoric skeletal remains from Barn Owl Cave.

Urocyon cinereoargenteus (Schreber, 1775)

Gray Fox

Fish Springs records. None.

Regional records. 5: Utah, Juab County, between Callao and Trout Creek (UMNH); Tooele County, 5 mi S Gold Hill (UMNH); Nevada, White Pine County, Snake Creek (MVZ),

Lexington Creek (MVZ), Stella Lake (UMNH). As a widespread habitat generalist (Fritzell and Haroldson 1982) with nearby regional specimen records, this species may occur on the refuge.

Vulpes macrotis Merriam, 1888

Kit Fox

Fish Springs records. 6: (UMNH).

Regional records. 10: Utah: Juab County, Topaz Mountain (UMNH), 1 mi SW Callao (UMNH); Millard County, Swasey Reservoir (BYU), 28 mi E NV border (BYU), Desert Experimental Range Station (UMNH); Tooele County, Dugway Valley (UMNH), 5 mi NE Granite Mountain (UMNH), 2 mi S Simpson Springs (UMNH); Nevada, Elko County, 3 mi E Silverzone Pass (UMNH); White Pine County, near Ely (BYU). Kit fox are common throughout the region as a small predator of rodents (Egoscue 1962).

Vulpes vulpes (Linnaeus, 1758)

Red Fox

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 1: Utah, Millard County, Garrison (UMNH). The Garrison specimen is the only Arctos record for this species within the eastern Great Basin, and distribution maps exclude it from the region (Barnes 1927; Durrant 1952; Hall 1981). As such, it's occurrence at Fish Springs seems highly unlikely.

Mustelidae (2 species documented from the refuge)

Neogale frenata (Lichtenstein, 1831)

Long-tailed Weasel

Fish Springs records. 1: (UMNH).

Regional records. 9: Utah, Juab County, 3 mi N Nephi (UMNH); Millard County Deseret (MVZ), Pavant Range, Robins Valley (UMNH); Tooele County, Little Granite Mountain (UMNH), 4 mi N Wig Mountain (UMNH), Cedar Mountains (UMNH); Nevada, Elko County, East Humboldt Range, Jerry Crab Spring (UMNH); White Pine County, Baker Creek (MVZ), Steptoe Creek (MSB). This species is common throughout the region.

Taxidea taxus (Schreber, 1777)

American Badger

Fish Springs records. 2: (UMNH).

Regional records. 10: Utah, Juab County, 2 mi W Trout Creek (UMNH); Millard County, Conger Springs (BYU), 4 mi W Deseret (MVZ); Tooele County, Ibapah (UMNH), 8 mi SW Simpson Spring (UMNH), Wig Mountain (UMNH); Nevada, Elko County, Montello (MVZ), 25 mi S Wells (UMZM); White Pine County, 7 mi W Utah State line (UMNH), Lehman Creek (MVZ). This species is common throughout the region.

Mephitidae (2 species documented from the refuge)

Mephitis mephitis (Schreber, 1776)

Striped Skunk

Fish Springs records. 4: (UMNH).

Regional records. 6: Utah, Millard County, 1 mi S Deseret (UMNH), Black Rock (UMNH); Tooele County, 0.5 mi N Granite Mountain (UMNH); 4 mi N Camelback Mountain (UMNH); Nevada, Elko County, Mary's River, 26 mi N Deeth (MVZ); White Pine County, Home Farm, 2.4 km N, 5.2 km W Baker (UMNH). This species is common throughout the region.

Spilogale gracilis Merriam, 1890

Western Spotted Skunk

Fish Springs records. 3: (UMNH).

Regional records. 9: Utah, Juab County, Callao (BYU), Deep Creek Range, Granite Creek Canyon (UMNH), above junction of Birch and Trout creeks (UMNH); Millard County, Confusion Range (UMNH), 16 mi N Deseret (MVZ); Tooele County, Granite Peak (BYU), Dugway Valley (UMNH); Nevada, Elko County, Ruby Mountains, vicinity of Flyn Spring (UMNH); White Pine County, McGill (UMZM). This species is common throughout the region.

Procyonidae (2 species, 1 of which is documented from the refuge)

Bassariscus astutus (Lichtenstein, 1830)

Ringtail

Fish Springs. 1: (UMNH).

Regional records. 4: Utah, Juab County, Deep Creek Mountains, Birch Creek Canyon (UMNH), mouth of Granite Creek Canyon (UMNH), mouth of Red Cedar Canyon (UMNH), Tooele County, South Willow Canyon (BYU); Nevada, White Pine County, Baker (UMNH). This species is likely common in rock outcrops along the eastern margin of Fish Springs Range adjacent to the refuge.

Procyon lotor (Linnaeus, 1758)

Raccoon

Fish Springs. No specimen records; one recent iNaturalist record (2019; https://www.inaturalist.org/observations/27973320).

Regional record. 1: Utah, Tooele County, Oquirrh Mountains, Middle Canyon (UMNH). Historically, the raccoon was considered rare in Utah (Durrant 1952), and distribution maps have excluded it from the region (Barnes 1927; Hall 1946, 1981; Zeveloff 1988). The specimen from Tooele County, collected in 1989, is the only Arctos record from

the eastern Great Basin. In recent decades raccoons have expanded into western Utah as documented from state trapping records (Kamler *et al.* 2003). The 2019 iNaturalist record strongly suggests that this species either has or may become established at Fish Springs.

Perissodactyla

Equidae (1 regional species not documented from the refuge)

Equus caballus Linnaeus, 1758

Horse (feral)

Fish Springs records. None.

Regional records. none. Although there are no regional specimen records, feral horses are common in the region, with nearby herds that are managed by the US Bureau of Land Management (https://www.blm.gov/programs/wild-horse-and-burro/herd-management/herd-management-areas/utah). At least occasionally, feral horses likely occur at Fish Springs.

Artiodactyla

Antilocapridae (1 species documented from the refuge)

Antilocapra americana (Ord, 1815)

Pronghorn

Fish Springs records. No recent specimen records.

Regional records. 4: Utah, Millard County, Desert Experimental Range Station (UMNH); Tooele County, 8 mi SW Simpson Spring (UMNH), 5 mi N Camelback Mountain (UMNH), Dugway Valley (UMNH). Pronghorn are frequently observed along the western margin of the refuge (USFWS 2004). Prehistoric skeletal remains were reported from Barn Owl Cave (Madsen 1982).

Cervidae (1 species documented from the refuge)

Odocoileus hemionus (Rafinesque, 1817)

Mule Deer

Fish Springs records. No recent specimen records.

Regional records. 8: Utah, Juab County, 5 mi S Nephi (BYU); Millard County, 2 mi N Oak City (BYU); Tooele County, Candy Creek, Cedar Mountains (UMNH), Benmore Guard Station (UMNH); Nevada, Elko County, Ruby Mountains, Lamoille Canyon (UMNH); White Pine County, Lehman Creek (MVZ), Baker Creek (MVZ), Duck Creek Valley (MSB). Deer reportedly occur on or near the refuge during summer and autumn (USFWS 2004). Prehistoric skeletal remains were reported from Barn Owl and Crab Caves (Madsen 1982).

Bovidae (1 species documented from the refuge)

Ovis canadensis Shaw, 1804

Bighorn Sheep

Fish Springs records. No recent specimen records.

Regional records. 5: Utah, Tooele County, Granite Mountain (UMNH), Lakeside Mountains (UMNH); Nevada, White Pine County, Hendry Creek (MVZ), Baker Creek (MVZ), Stella Lake (MVZ). All regional records are based on salvaged specimens (heavily weathered partial skulls). Prehistoric skeletal remains were reported from Barn Owl Cave (Madsen 1982). Overhunting and/or disease transmission from domestic sheep probably eradicated bighorn sheep locally by the early 20th century (Besser *et al.* 2012; Cassirer *et al.* 2017) however regional reintroduction efforts (Utah Division of Wildlife Resources 2018) may eventually lead to their reestablishment in the Fish Springs Range.

Rodentia

Sciuridae (6 species, 1 of which is documented from the refuge)

Ammospermophilus leucurus (Merriam, 1889)

White-tailed Antelope Squirrel

Fish Springs records. 28: (BYU and UMNH).

Regional records. 12: Juab County, Fish Springs Range (UMNH), junction Snake Valley and Granite Creek roads (UMNH), 5.5 mi S, 7.8 mi E Callao (MVZ), 6.7 mi S, 5.3 mi E Callao (MSB), 1.5 mi N Topaz Mountain (UMNH); Millard County, Tule Valley (UWYMV), Marjum Pass (UMNH), Gandy Salt Marsh (BYU); Tooele County, Gold Hill (BYU), Granite Mountain (UMNH), Camelback Mountain (UMNH); Nevada, White Pine County, Baker (UMNH). This species is common and widespread on the refuge, particularly in mixed shrublands (Table 2).

Marmota flaviventris (Audubon and Bachman, 1841).

Yellow-bellied Marmot

Fish Springs records. None.

Regional records. 5: Utah, Juab County, 4 mi W Eureka (UMNH); Tooele County, 9 mi S Vernon (BYU), Stansbury Mountains (UMNH); Nevada, Elko County, Lamoille Creek (MVZ) White Pine County, Baker Creek Canyon (UMNH). Reported (sighting) from Granite Creek Canyon, Deep Creek Range 35 km west of Fish Springs (Shippee and Egoscue 1958). Marmots may be present in the Fish Springs Range west of the refuge.

Neotamias dorsalis (Baird, 1855)

Cliff Chipmunk

Fish Springs records. None.

Regional records. 9: Utah, Juab County, Deep Creek Mountains, 4.0 km E, 4.4 km S Ibapah Peak (BYU), mouth of Indian Farm Canyon (UMNH), Cherry Creek (UMNH); Millard County, 1 mi N Sinbad Spring (UMNH); Tooele County, 1 mi N Gold Hill (UMNH), Granite Peak (UMNH), Indian Springs (UMNH); Nevada, Elko County, Ferguson Hills (UMNH); White Pine County, Mt. Moriah (MVZ). This species generally occurs in rocky areas in shrubland or woodland habitats (Hart 1992); it may occur in the western-most portions of the refuge.

Neotamias minimus (Bachman, 1839).

Least Chipmunk

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 14: Utah, Juab County, Deep Creek Mountains, Granite Creek Canyon (UMNH), mouth of Indian Farm Canyon (UMNH); Millard County, Gandy (MVZ). Pavant Range (UMNH); Tooele County, Clifton Flat (UMNH), NW of Granite Peak (UMNH), 8 mi SW Wig Mountain (UMNH), 3 mi W Camelback Mountain (UMNH); Nevada, Elko County, Dolly Varden Mountains (UMNH), Cherry Creek Mountains (DMNS), Spruce Mountain (HSUVM); White Pine County, Shellbourne Pass (MVZ), Steptoe Valley (MVZ), Strawberry Creek Canyon, Snake Range (UMNH). This species occurs in shrubland, generally in areas dominated by sagebrush (Verts and Carraway 2001). It may occur in mixed shrub habitat on the western portion of the refuge.

Urocitellus mollis (Kennicott, 1863)

Piute Ground Squirrel

Fish Springs records. None.

Regional records. 12: Utah, Juab County, 5 mi SE Fish Springs (UMNH), 0.7 mi N Black Rock Hills (UMNH), Thomas Range (UMNH), 2.5 mi E Topaz Mountain (UMNH); Millard County, Deseret (MVZ), 15 mi S, 6 mi E Garrison (UMNH); Tooele County, 2 mi S Ibapah (UMNH), 5.7 mi W Simpson Springs (UMNH); Nevada, Elko County, Currie (UWBM), Montello (MSB); White Pine County, Steptoe Valley (MVZ), Spring Valley (MVZ). This species may be present throughout much of Fish Springs Flat in the eastern portion of the refuge. It is dormant during most of the year, but active during the day above ground from late winter to late spring (Rickart 1987).

Otospermophilus variegatus (Erxleben, 1777)

Rock Squirrel

Fish Springs records. None.

Regional records. 10: Utah, Juab County, 7 mi S Callao (UWBM), Granite Creek Canyon, Deep Creek Mountains (UMNH), Ferner Valley (MSB); Millard County, Oak Creek Canyon (UMNH), Meadow (UMNH); Tooele County, Death Canyon, Simpson Mountains (UMNH), Little Valley Ranger Station, Sheeprock Mountains (UMNH); Nevada, White Pine County, Cherry Creek (MVZ), Cleve Creek, Schell Creek Range (MVZ). Snake Creek, Snake Range (UNR). This is a common regional species that may occur in the Fish Springs Range west of the refuge.

Heteromyidae (6 species, 3 of which are documented from the refuge)

Dipodomys microps (Merriam, 1904)

Chisel-toothed Kangaroo Rat

Fish Springs records. 55: (BYU and UMNH).

Regional records. 12: Utah, Juab County, south side

Table 3. Number of small mammal (rodent and shrew) occurrence records and percent relative abundance of species from trapping surveys at Fish Springs National Wildlife Refuge, Juab County, Utah, USA. Records from BYU (1992) and UMNH (1940, 2002–2018).

	1940	1992	2002–2003	2012	2015–2018
Species	***n* (% total)**	***n* (% total)**	***n* (% total)**	***n* (% total)**	***n* (% total)**
Ammospermophilus leucurus	1 (2.7)	6 (5.1)	7 (3.8)	9 (6.1)	2 (2.0)
Chaetodipus formosus	0	0	16 (8.7)	2 (1.3)	3 (3.1)
Dipodomys microps	4 (10.8)	18 (11.4)	21 (11.4)	10 (6.8)	6 (6.1)
Dipodomys ordii	5 (13.5)	4 (3.4)	3 (1.6)	1 (0.7)	1 (1.0)
Microtus montanus	4 (10.8)	2 (1.7)	11 (6.0)	8 (5.4)	20 (20.4)
Mus musculus	0	8 (6.8)	18 (9.8)	13 (8.9)	0
Neotoma lepida	2 (5.4)	0	4 (2.2)	9 (6.1)	3 (3.1)
Onychomys leucurus	0	1 (0.9)	1 (0.5)	0	0
Peromyscus crinitus	0	0	7 (3.8)	15 (10.1)	0
Peromyscus maniculatus	20 (54.1)	45 (38.5)	49 (26.6)	37 (25.0)	32 (32.7)
Reithrodontomys megalotis	1 (2.7)	32 (27.4)	26 (14.1)	32 (21.6)	18 (18.4)
Sorex preblei	0	0	0	0	1 (1.0)
Sorex vagrans	0	1 (0.9)	21 (11.4)	12 (8.1)	12 (12.2)
Total	37	117	184	148	98

Topaz Mountain (CSULB), junction Granite Creek Road and Snake Valley Road (BYU), Joy (BYU); Millard County, Tule Valley (UWYMV), 1 mi SE Gandy (MVZ); Tooele County, Dugway Proving Ground (MSB), Dugway Valley (UWBM), 3 mi SW Gold Hill (UMNH); Nevada, Elko County, Leppy Peak (UMNH); White Pine County, Steptoe Valley (MVZ), Spring Valley (MSB), Baker (UMNH). This widespread species is and abundant on the refuge, particularly in areas with *Atriplex* spp. (Table 2).

Dipodomys ordii Woodhouse, 1853

Ord's Kangaroo Rat

Fish Springs records. 13: (BYU and UMNH).

Regional records. 14: Utah, Juab County, Sand Pass (UMNH), Callao (BYU), Topaz Mountain (CSULB), Joy (BYU), Trout Creek (MVZ); Millard County, Tule Valley (UWYMV), Gandy Salt Marsh (BYU); Tooele County, Dugway Proving Ground (MSB), 1 mi W Five Mile Hill (UMNH); Nevada, Elko County, Salt Spring (MVZ), Shanty Town (UMNH); White Pine County, Mt. Moriah (MVZ), Spring Valley (MSB), Baker (UMNH). Although regionally widespread, this species is much less abundant on the refuge than *D. microps* (Table 3).

Microdipodops megacephalus Merriam, 1891

Dark Kangaroo Mouse

Fish Springs records. None.

Regional records. 15: Utah, Juab County, 3 mi SSW Fish Springs (UMNH), 5.5 mi S, 7.8 mi E Callao (MSB), 6.7 mi S, 5.3 mi E Callao (MSB), 7.7 mi N, 2.7 mi E Callao (MVZ), 4.5 mi NE Trout Creek (UMNH), 4 mi SW Trout Creek (UMNH); Millard County, Tule Valley (UWYMV), 5 mi S Gandy (MVZ); Tooele County, 8 mi E Granite Mountain (UMNH), 3 mi N Granite Mountain (UMNH), 2 mi NE Camelback Mountain (UMNH); Nevada, Elko County, Cobre (MVZ), Ruby Lake National Wildlife Refuge (UMNH); White Pine County, 7 mi SW Osceola (MVZ), 4 mi S Shoshone (MVZ). This is a Great Basin endemic species of conservation concern. Populations have been declining, and Fish Springs is near the eastern limit of the geographic range (Anderson *et al.* 2013; Light *et al.* 2013). Kangaroo mice may occur on the refuge in areas with suitable soil and plant cover.

Chaetodipus formosus (Merriam, 1899)

Long-tailed Pocket Mouse

Fish Springs records. 26: (BYU and UMNH).

Regional records 12: Utah, Juab County, 3 mi W Callao (UMNH), junction Granite Creek and Snake Valley roads (UMNH), Sand Pass (UMNH), Topaz Mountain (CSULB); Millard County, Swasey Springs (UMNH), Gandy Salt Marsh (BYU), Black Rock (UMNH); Tooele County, 3.0 km E, 0.4 km S Young Peak, Deep Creek Range (BYU), Dugway Mountains (UMNH); Nevada, Elko County, Goshute Mountains (MVZ), Leppy Peak (UMNH); White Pine County, Mt. Moriah (MVZ). This species is regionally abundant, and common in mixed arid shrubland along the western margin of the refuge (Table 2).

Perognathus longimembris (Coues, 1875)

Little Pocket Mouse

Fish Springs records. None.

Regional records. 15: Utah, Juab County, 1 mi S Callao (UMNH), 0.25 km E, 0.20 km S junction Granite Creek Road and Snake Valley Road (BYU), Trout Creek (UMNH), Leland Harris Spring (BYU), Sand Pass (UMNH), Joy (BYU); Millard County, Tule Valley (UWYMV), Desert Experimental Range (MSB); Tooele County, Table Mountain (UMNH), 4 mi NW Camelback Mountain (UMNH), 6 mi NE Granite Mountain (UMNH); Nevada, Elko County, 16 mi S Montello (UMNH), Ruby Lake National Wildlife Refuge (UMNH); White Pine County, Mt. Moriah (MVZ), 1.6 mi N Baker (MVZ). Regionally common, this species may occur in areas on the refuge with suitable soil and plant cover.

Perognathus mollipilosus (Coues, 1875)

Great Basin Pocket Mouse

Fish Springs records. None.

Regional records. 15: Utah, Juab County, Callao (BYU), Deep Creek Mountains, Granite Creek Canyon (UMNH), Joy (BYU); Millard County, House Range (UMNH), Marjum Pass, House Range (BYU), 7 mi SW Skull Rock Pass (MVZ); Tooele County, Dugway Mountains (UMNH), Simpson Mountains (UMNH), Clifton Flat (UMNH); Nevada, Elko County, Dolly Varden Mountains (UMNH), Spruce Mountain (MVZ), East Humboldt Range (UMNH); White Pine County, Mt. Moriah (MVZ), Schellbourne Pass (MVZ), Spring Valley (UMNH). This species occurs throughout the Great Basin and is common in arid habitat with sandy soil where sagebrush (*Artemisia* spp) is dominant (Verts and Kirkland 1988); it may occur in the northwestern portion of the refuge.

Geomyidae (1 species documented from the refuge)

Thomomys bottae (Eydoux and Gervais, 1836)

Botta's Pocket Gopher

Fish Springs records. 30: (BYU and UMNH).

Regional records. 13: Utah, Juab County, Sand Pass (UMNH), Callao (UMNH), Trout Creek (UMNH); Millard County, Tule Valley (UWYMV), Swasey Spring (UMNH), Marjum Pass (UMNH); Tooele County, Dugway Range (UMNH), Five Mile Hill (UMNH), Granite Mountain (UMNH); Nevada, Elko County, Pilot Creek (UMNH); White Pine County, Mt. Moriah (MVZ), 2.5 mi E Baker (MVZ), Snake Creek Canyon (UMNH). Fish Springs is the type locality for the subspecies *T. bottae bonnevillei* Durrant,1946. This species is common throughout the region within which there are several recognized local subspecies (Patton 2005).

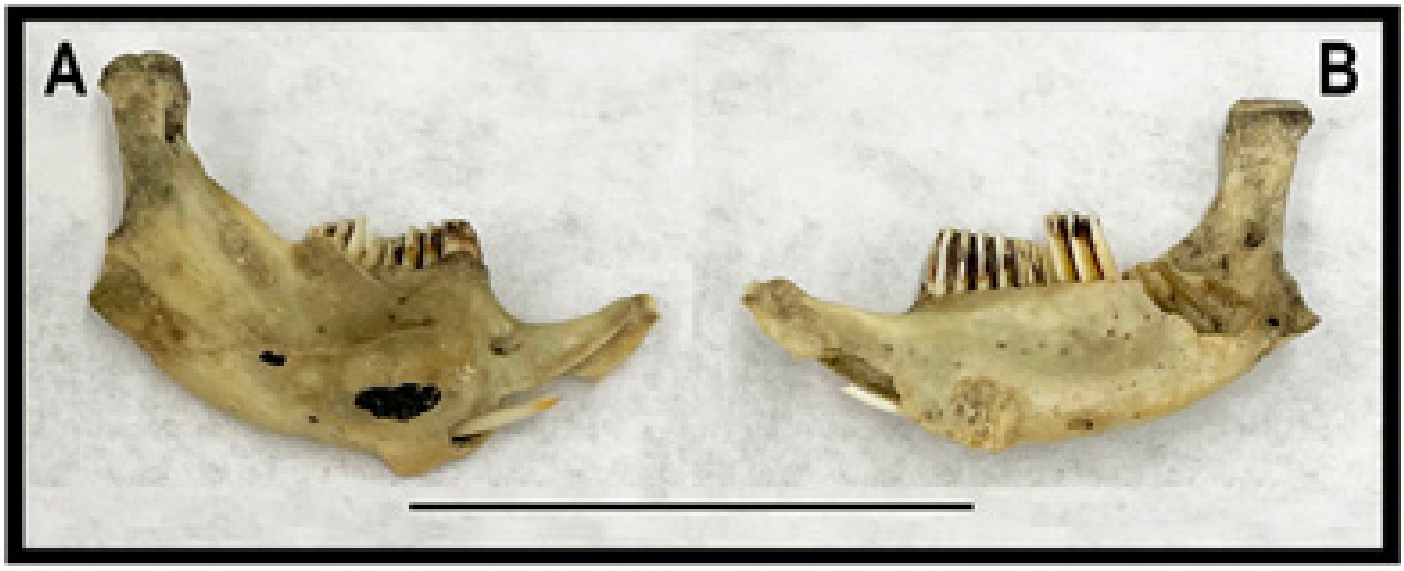

Figure 3. A) Labial and B) lingual views of a partial muskrat right hemimandible from Barn Owl Cave, Fish Springs National Monument dated between *ca*. 4000 and 1800 BP. UMNH archaeological collection (42Jb25 AS.78.28.5.11). Scale bar = 3 cm.

Cricetidae (10 species, 6 of which are documented from the refuge).

Lemmiscus curtatus (Cope, 1868)

Sagebrush Vole

Fish Springs records. None.

Regional records. 12: Utah, Juab County, Deep Creek Mountains, mouth of Indian Farm Canyon (UMNH), Queen of Sheba Canyon (UMNH), 2.7 km N, 1.3 km E Indian Springs (UMNH); Millard County, Marjum Pass, House Range (BYU); Tooele County, 7 mi SW Gold Hill (UMNH), Simpson Springs (UMNH); Nevada, Elko County, Dolly Varden Mountains (UMNH), Cobre (MVZ), 20 mi N Wells (UMNH); White Pine County, Mt. Moriah (MVZ), Schell Creek Range MVZ), 2 mi NW Sacramento Pass (UMNH). This species is usually found in association with sagebrush (*Artemisia* spp) in areas with well-drained soils (Carroll and Genoways 1980). It may occur in mixed shrubland in the northwestern part of the refuge that includes a portion of the Fish Springs Range.

Microtus longicaudus (Merriam, 1888)

Long-tailed Vole

Fish Springs records. None.

Regional records. 15: Utah, Juab County, Deep Creek Mountains, Indian Farm Canyon (UMNH), Tom's Creek (HSUVM), Granite Creek Canyon (UMNH); Millard County, Sinbad Spring (UMNH), 4 mi E Oak City (UMNH), Fillmore (BYU); Tooele County, Simpson Springs (UMNH), Indian Springs (UMNH), Hard to Beat Canyon (BYU); Nevada, Elko County, 12.5 mi SW Wendover (UMNH), Debbs Creek, Pilot Range (BYU), Spruce Mountain (MVZ); White Pine County, Mt. Moriah (MVZ), Snake Range, mouth of Pole Canyon (UMNH), south fork Big Wash (UMNH). This species may occur in mesic habitat or arid grassland on the refuge.

Microtus montanus (Peale, 1848)

Montane Vole

Fish Springs records. 55: (BYU and UMNH).

Regional records. 10: Utah, Juab County, Callao (UMNH); Millard County, 5 mi S Garrison (MVZ); Tooele County, 1 mi E Ibapah (UMNH), 7.5 mi E Granite Mountain (UMNH); Nevada, Elko County, Ferguson Springs (UMNH), Salt Spring (MVZ), Currie (UWBM); White Pine County, Baker (UMNH), Cleveland Ranch (MVZ), 7 mi SW Osceola (MVZ). Montane voles are abundant on the refuge, but largely restricted to mesic habitat (Table 2).

Ondatra zibethicus (Linnaeus, 1766)

Muskrat

Fish Springs records. 88: (BYU and UMNH**).**

Regional records. 7: Utah, Juab County, Mills (UMNH); Millard County, 10 mi N Deseret (MVZ), 5 mi S Garrison (MVZ); Tooele County, Ibapah (UMNH), Horseshoe Springs (BYU); Nevada, Elko County, Ruby Lake (MVZ); White Pine County, Lehman Creek (UMNH). As a commercially valuable furbearer, this species has been introduced to many areas where it was not present historically (Hall 1946, 1981; Durrant 1952). The occurrence of muskrats at Fish Springs was first noted by Barnes (1927) where they were reported to have been introduced in 1925 (McCabe 1982; also see Bolen 1964), and therefore considered to be non-native (USFWS 2004). Introduced animals may have been sourced from the Bear River Migratory Bird Refuge where non-native muskrats from the eastern US were introduced to improve the size and quality of the pelts (Durrant 1952).

Despite the probable introduction of non-native muskrats to Fish Springs, skeletal material from Barn Owl Cave documents prehistoric occurrence of native muskrats (Figure 3; Madsen 1982). Muskrats are abundant on the refuge and are considered beneficial in maintaining suitable habitat for waterfowl (USFWS 2004).

Neotoma lepida (Thomas, 1893)

Desert Woodrat

Fish Springs records. 27: (BYU and UMNH).

Regional records. 15: Utah, Juab County, Deep Creek Range, Granite Creek Canyon (UMNH), Trout Creek (BYU), south side Topaz Mountain (CSULB); Millard County, Tule Valley (UWYMV), Swasey Spring (UMNH), Gandy Salt Marsh (BYU); Tooele County, Cane Spring (MSB), Granite Peak (UMNH), Dugway Valley (UWBM); Nevada, Elko County, Ferguson Hills (UMNH). 9.8 km S, 4.4 km W West Wendover (UMNH), 1.5 mi SW Currie (MVZ); White Pine County, Mt. Moriah (MVZ), South Snake Ridge (UNR), Baker (MVZ). This species is common throughout the region, but most often found in rocky habitat. Most specimens from the refuge were trapped in arid shrubland along the base of the Fish Springs Range (Table 2).

Onychomys leucogaster (Wied-Neuwied, 1841)

Northern Grasshopper Mouse

Fish Springs records. 2: (BYU and UMNH).

Regional records. 15: Utah, Juab County, 7 mi S Fish Springs (UMNH), Trout Creek (UMNH), Little Sahara near Cherry Creek Wash (BYU); Millard County, Tule Valley (UWYMV), 4 mi S Gandy (MVZ), 7 km S Oak City (MSB); Tooele County, 2 mi N Fish Springs Mountains (UMNH), Dugway Valley (UWBM), 3 mi SW Gold Hill (UMNH); Nevada, Elko County, Tecoma (MVZ), Cobre (MVZ), Davis Spring (UMNH); White Pine County, Baker (UMNH), Spring Valley (MSB), Steptoe Creek (MVZ). The species occurs throughout the region, but is much less common locally than most other rodent species (Table 3).

Peromyscus crinitus (Merriam, 1891)

Canyon Mouse

Fish Springs records. 32: (BYU and UMNH).

Regional records. 14: Utah, Juab County, 7 mi S Fish Springs (UMNH). Granite Creek Canyon, Deep Creek Mountains (UMNH), confluence of Birch and Trout creeks (UMNH); Millard County, Gandy Salt Marsh (BYU), Marjum Pass (UMNH), west of Meadow (UMNH); Tooele County, Dugway Mountains (UMNH), Granite Peak Range (UMNH), 1 mi SW Gold Hill (UMNH); Nevada, Elko County, Leppy Peak (UMNH), 9.8 km S, 4.4 km W West Wendover (UMNH); White Pine County, Mt. Moriah (MVZ), Schell Creek Range (MVZ), Cherry Creek (MVZ). This species is a habitat specialist that occurs on rocky slopes. The species is restricted to the mixed arid shrubland along the western margin of the refuge where it is common (Table 2).

Peromyscus maniculatus (Wagner, 1845)

Deer Mouse

Fish Springs records. 117 (BYU and UMNH).

Regional records. 15: Utah, Juab County, Fish Springs Range (UMNH), Sand Pass (UMNH), Trout Creek (BYU); Millard County, 4 mi S Gandy (MVZ), Swasey Spring (UMNH), 7 mi SW Skull Rock Pass (MVZ); Tooele County, Clifton Flat (UMNH), 18 mi SW Orr's Ranch (UMNH), Simpson Springs (MSB); Nevada, Elko County, Leppy Peak (UMNH), Dolly Varden Mountains (UMNH), Spruce Mountain (MVZ); White Pine County, Mt. Moriah (MVZ), Baker (UMNH), Pyramid Peak (UMNH). As an abundant ecological generalist, this is the only species that occurs in all habitats on the refuge and is the most abundant small mammal across all sampling periods (Tables 2 and 3).

Peromyscus truei (Shufeldt, 1885)

Pinyon Mouse

Fish Springs records. None. Included in the refuge species list (USFWS 2004) based on one record (below) from the adjacent Fish Springs Range.

Regional records 15: Utah, Juab County, Fish Springs Range (BYU), Indian Farm Canyon (BYU), confluence Birch and Trout creeks (UMNH); Millard County, Marjum Pass (BYU), 4 mi S Oak City (BYU), Desert Experimental Range Station (UMNH); Tooele County, 7 mi NE Granite Peak Range (UMNH), Dugway Proving Ground, Cane Spring (MSB), Simpson Springs (UMNH); Nevada, Elko County, Ferguson Hills (UMNH), Toano Peak (BYU), Debbs Creek, Pilot Peak (MVZ); White Pine County, Snake Creek Canyon (UNR), Schell Creek Range (MVZ), Cherry Creek (MVZ). The geographic range of pinyon mice is expanding in much of the intermountain west with the spread of pinyon-juniper woodland (Massey *et al.* 2017). The species is known from a nearby locality in the Fish Springs Range, and likely occurs on the northwestern margin of the refuge.

Reithrodontomys megalotis (Baird, 1857)

Western Harvest Mouse

Fish Springs records. 76: (BYU and UMNH).

Regional records 15: Utah, Juab County, Sand Pass (UMNH), 0.5 mi S Callao (UMNH), Granite Creek Canyon (UMNH); Millard County, Tule Valley (UWYMV), 1 mi SE Gandy (MVZ), Robinson Ranch (MVZ); Tooele County, Clifton Flat (UMNH), 7 mi NE Granite Peak Range (UMNH), Indian Springs (UMNH); Nevada, Elko County, Salt Springs (MVZ), Debbs Creek (BYU), Toano Peak (BYU); White Pine County, Mt. Moriah (MVZ), Baker (UMNH), Sacramento Pass (UMNH). Harvest mice are common throughout the region and occur in most habitats on the refuge (Table 2).

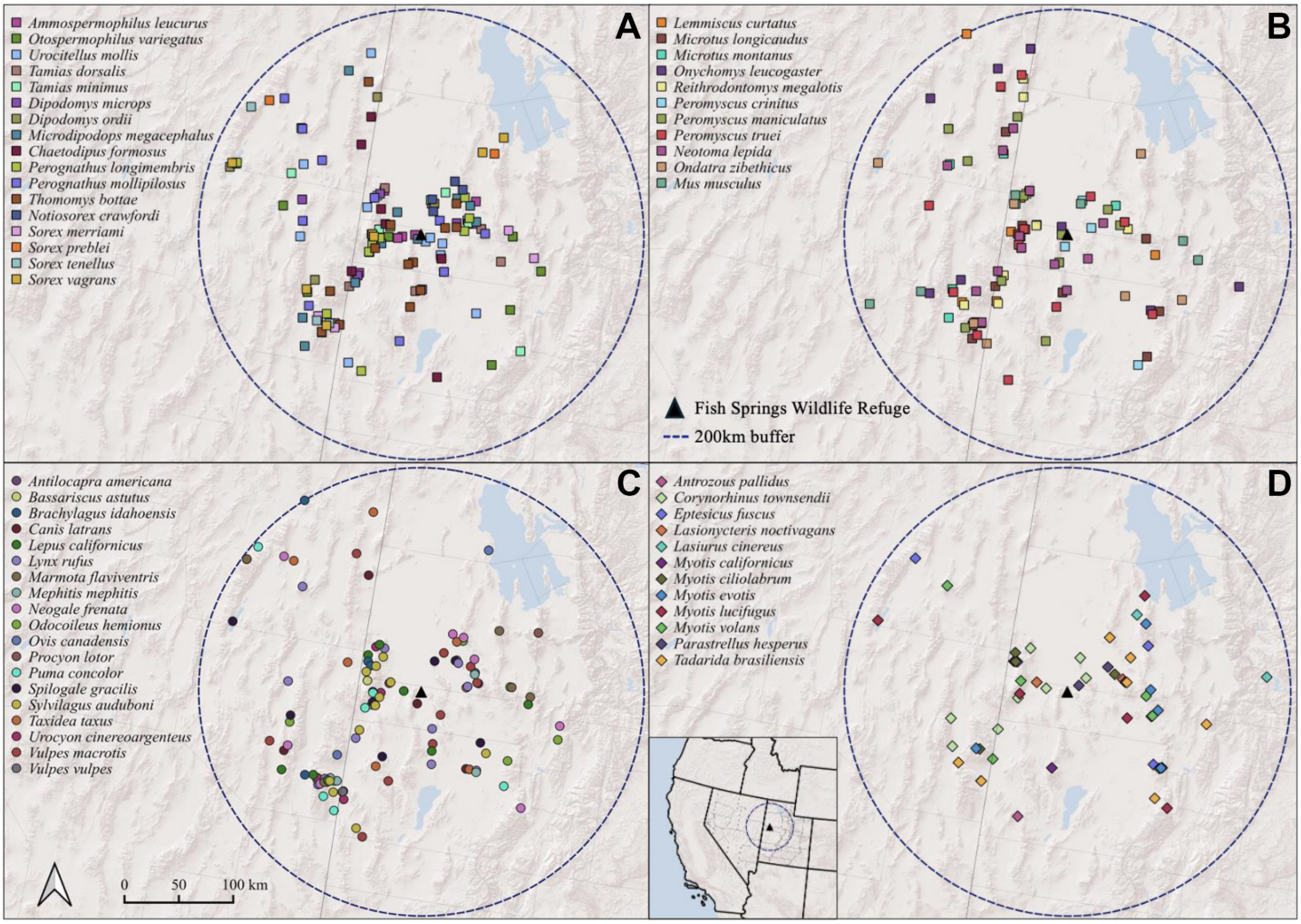

Figure 4. Maps of regional mammal specimen records from within 200 km of Fish Springs National Wildlife Refuge in western Utah (inset). A) small nonvolant species (shrews, small sciurid, heteromyid and geomyid rodents), B) small cricetid rodents, C) medium and large species (rodents, lagomorphs, ungulates and carnivorans), and D) bats.

Muridae (1 species documented from the refuge)

Mus musculus Linnaeus, 1758

House Mouse

Fish Springs records. 36: (BYU and UMNH).

Regional records 7: Utah, Juab County, Eureka (BYU), 4 mi W Mona (UMNH); Tooele County, Dugway Proving Ground (UMNH), Parish Ranch (UMNH); Nevada, Elko County, 3 mi N Jarbidge (UNR); White Pine County, Baker (UMNH), 5.5 mi SE Ely (MVZ). The earliest specimen records for house mice at Fish Springs are from 1992 (Table 3), but they may have been present much earlier, perhaps dating from increased human traffic in the early 20th century. The earliest regional specimens are from Baker [1929] and Ely (1930) in Nevada, and from Ibapah [1942] and Mona [1951] in Utah (listed above). Although regional records are sparse, house mice have been relatively abundant at Fish Springs, occurring in disturbed and heavily managed habitat, as well as in areas dominated by native vegetation (Table 2). However, there appears to have been a population crash prior to the most recent survey period when none were recorded (Table 3).

Lagomorpha

Leporidae (3 species, 2 of which are documented from the refuge)

Brachylagus idahoensis (Merriam, 1891)

Pygmy rabbit

Fish Springs records. None. Included in the Fish Springs species list (USFWS 2004) but there are no vouchered records.

Regional records. 4: Utah, Tooele County, Ibapah (UMNH), 3 mi. SE Ibapah (UMNH); Nevada, Elko County, 10 mi N Wells (UMNH); White Pine County, Spring Valley (UMNH). This species is closely associated with dense shrub cover dominated by big sagebrush (*Artemisia tridentata*) in areas with sandy soil (Green and Flinders 1980; Gabler *et al.* 2001). Although present in the region, its occurrence in or near the refuge is highly unlikely given the lack of preferred sagebrush habitat.

Sylvilagus audubonii (Baird, 1858)

Desert Cottontail

Fish Springs records. 2: (BYU).

Table 4. Mammal species without vouchered records from Fish Springs NWR, but either reported to occur there or having some likelihood of occurrence based on vouchered records elsewhere and presence of suitable habitat.

Species	Included in FSNWR species list	Nearest voucher	Voucher distance (km)	Habitat suitability	Likelihood of occurrence
Notiosorex crawfordi	no	BYU:Mamm:33208	18	high	high
Sorex merriami	no	MSB:Mamm:102731	75	moderate	moderate
Sorex tenellus	no	UMNH:Mamm:37371	40	moderate	moderate
Tadarida brasiliensis	yes	UMNH:Mamm:27275	50	high	high
Antrozous pallidus	yes	UMNH:Mamm:27248	25	high	high
Corynorhinus townsendii	yes	UMNH:Mamm:23958	20	high	high
Eptesicus fuscus	no	UMNH:Mamm:27232	25	high	high
Lasiurus cinereus	no	BYU:Mamm:36740	35	moderate	moderate
Myotis californicus	yes	UMNH:Mamm:4424	55	high	high
Myotis evotis	yes	BYU:Mamm:18086	75	moderate	moderate
Myotis thysanodes	yes	BYU:Mamm:10439	180	moderate	low
Myotis yumanensis	yes	BYU:Mamm:41155	140	moderate	low
Puma concolor	no	UMNH:Mamm:5466	40	moderate	moderate
Urocyon cinereoargenteus	no	UMNH:Mamm:25326	35	moderate	moderate
Vulpes vulpes	yes	UMNH:Mamm:42254	110	low	low
Procyon lotor	no	UMNH:Mamm:28778	120	moderate	high
Marmota flaviventris	no	BYU:Mamm:5379	80	low	low
Tamias dorsalis	no	UMNH:Mamm:26431	25	high	high
Tamias minimus	yes	UMNH:Mamm:26423	30	moderate	moderate
Urocitellus mollis	yes	UMNH:Mamm:24851	8	high	high
Otospermophilus variegatus	no	UWBM:Mamm:42860	35	moderate	moderate
Microdipodops megacephalus	yes	UMNH:Mamm:24772	5	high	high
Perognathus longimembris	yes	UMNH:Mamm:25779	25	high	high
Perognathus mollipilosus	yes	BYU:Mamm:3099	25	high	high
Lemmiscus curtatus	no	UMNH:Mamm:25564	40	moderate	moderate
Microtus longicaudus	no	UMNH:Mamm:26856	40	moderate	moderate
Peromyscus truei	yes	BYU:Mamm:14698	4	moderate	high
Brachylagus idahoensis	yes	UMNH:Mamm:7862	50	low	low

Regional records. 11: Utah, Juab County, Callao (UMNH), Mayfield Ranch (UMNH), Snake Valley (UMNH); Millard County, Gandy Salt Marsh (BYU), 6 mi N Delta (UMNH), Desert Experimental Range Station (UMNH); Tooele County, 2 mi W Willow Spring (UMNH), Clifton Flat (UMNH), 6 mi S Ibapah (UMNH); Nevada, White Pine County, SW of Baker (BYU), Snake Creek (UNR). This species is likely very common in desert shrubland habitat on the refuge. Prehistoric skeletal remains were reported from Barn Owl Cave (Madsen 1982).

Lepus californicus Gray, 1837

Black-tailed Jackrabbit

Fish Springs records. 1: (BYU).

Regional records. 8: Utah, Juab County, Boyd's Ranch (UMNH). 6 mi SW Eureka (MSB); Millard County, E of House Range (UMNH); Tooele County, 4 mi NW Ibapah (BYU), 4 mi E Camelback Mountain (BYU); Nevada, White Pine County, 7.3 km W Baker (BYU), Willard Creek (UMNH), Duck Creek Valley (MSB). Madsen (1982) reported prehistoric skeletal remains from Barn Owl Cave and Crab Cave (Madsen 1982). This species is commonly observed on the refuge.

Results and Discussion

Legacy specimen records together with specimens from recent survey work document a total of 33 mammal species from Fish Springs. An additional 27 species were documented from regional specimen records within the eastern Great Basin region (Figure 4). This second group includes species with variable likelihood of occurrence on the refuge as addressed in the species accounts above and discussed below. Additional species include two bats (*Myotis thysanodes* and *M. yumanensis*) that are on the refuge mammal species list (USFWS 2004) but have no specimen records from within the eastern Great Basin, and one species (feral *Equus caballus*) that lacks regional specimen records but has been documented on or near the refuge.

Data quality and survey thoroughness. Our study is based almost entirely on records of occurrence from museum specimens. In analyses of relative abundance of rodents and shrews across habitat type and years (Tables 1 to 3), we included some individuals that were trapped and released after identification, but distribution records for all species are based on voucher specimens.

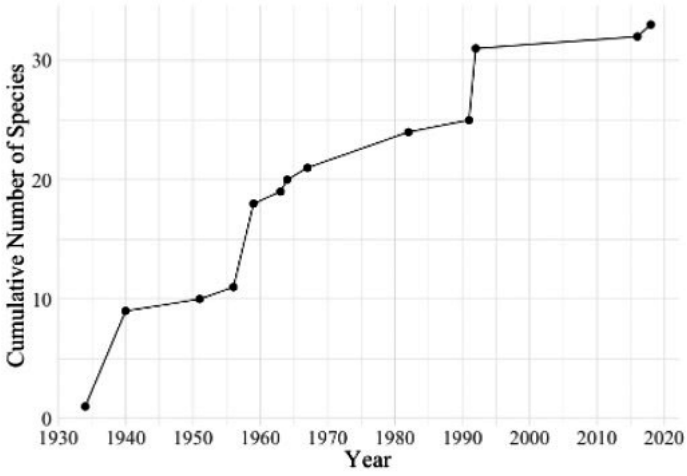

Figure 5. Cumulative number of mammal species documented over time at Fish Springs National Wildlife Refuge based on museum specimen records.

Over the course of 85 years (1934 to 2018), specimen records have documented 33 mammal species on the Fish Springs Refuge, including some that were present in the past but may not occur there now (*e. g.*, *Ovis canadensis*). A species accumulation curve across years (Figure 5) shows a stepwise increase due to the addition of multiple species during separate surveys, but it does not exhibit an extended asymptote indicating that future surveys would very likely document additional species.

There are 27 species that either belong to the regional fauna or have been reported as occurring on the refuge but are not represented by vouchers (Table 4). More than half (15) appear in a list of mammals for the refuge in the Comprehensive Conservation Plan (USFWS 2004). Although there is no reference to the criteria for placement on the list, they presumably included sightings of diurnal species, presence of sign (tracks or scat), use of bat detectors, capture and release after field identification, and specimen records from near the refuge. The nearest vouchered regional records for these species, along with our assessment of habitat suitability on the refuge (as summarized in species accounts) provide the basis for evaluating the likelihood of occurrence at Fish Springs (Table 4).

Recent survey efforts have utilized standard small mammal traps that are most effective in targeting rodents and the most common shrew species. In the future, a wider range of survey techniques would be most productive, including pitfall traps that are more effective in capturing many species of shrews (Williams and Braun 1983; Stephens and Anderson 2014), acoustical surveys of bats together with mist-netting for species identification and collection of voucher specimens, and camera trapping to document large species (carnivorans and ungulates).

Habitat disturbance and temporal trends. Fish Springs has a long history of disturbance, both prior to its establishment as a wildlife refuge and afterward due to wetland engineering and ongoing management practices. Small mammals most abundant in riparian habitat (*e. g.*, *Microtus montanus*, *Mus musculus*, and *Sorex vagrans*; Table 2) probably benefited from these habitat modifications, whereas xeric species may have faced reductions in available habitat. Trapping surveys conducted from 1940 to 2018 (Table 3) show little change across time in relative abundance of the most common habitat generalist, *Peromyscus maniculatus*. Apparent variation in abundance of species most common in mesic habitat (*Sorex vagrans* and *Microtus montanus*) and arid shrubland (*Chaetodipus formosus* and *Peromyscus crinitus*) is probably an artifact of uneven survey effort across habitats.

Unfortunately, there is very little information on small mammals at Fish Springs before the refuge was established, only the results of a brief trapping survey by Stephen Durrant and Henry Setzer in June 1940 (Table 3). One noteworthy result of this early survey was nearly equal abundance of both *Dipodomys* species that were trapped together in habitat dominated by halophytic shrubs. Grayson (2011) highlighted *D. microps* and *D. ordii* as particularly good indicators of local habitat conditions. The former species is largely folivorous consuming succulent leaves of halophytic shrubs, particularly *Atriplex* (Kenagy 1973; Hayssen 1991), whereas the latter is granivorous and more of a habitat generalist (Garrison and Best 1990). Both species have coexisted on the refuge across time, but the greater abundance of *D. microps* in recent surveys reflects the extensive presence of halophytes, whereas *D. ordii* has remained relatively uncommon perhaps as a result of refuge management practices.

Non-native house mice were first collected on the refuge in 1992, but were likely introduced much earlier. As a non-native commensal species, feral house mice in temperate locations generally require access to human food resources associated with agriculture, as well as artificial shelter from seasonal weather extremes (Kaufman and Kaufman 1990, 2018). At Fish Springs, house mice were documented across a broad range of habitats (Table 2) and were most abundant around spring margins where geothermal heat may sustain continual plant growth and extend reproductive activity (Negus *et al.* 1986). House mice were relatively common from 1992 through 2012, but appear to have suffered a population crash prior to the most recent surveys (2015 to 2018) when none were recorded (Table 3). This may have resulted from extremely cold winter weather between 2012 and 2014.

Management. With the enactment of National Wildlife Refuge System Improvement Act of 1997, a Comprehensive Conservation Plan (CCP) was developed for the Fish Springs Refuge focusing on providing habitat for maximum wildlife diversity (USFWS 2004). Much of this plan focused on the Harrison Unit, the northwestern portion of the refuge receiving the discharge of North Spring (Figure 1). Goals of the CCP included restoring habitats on a portion of the refuge to conditions similar to those that existed prior to wetland engineering, and implementing ongoing monitoring of vegetation and wildlife.

The isolation of Fish Springs has limited the type of disturbance associated with heavy visitation and human encroachment that has impacted more accessible wildlife refuges. As such, Fish Springs offers a unique perspective on the environmental effects of targeted wildlife management practices. Our results suggest that environmental disturbance from decades of focused waterfowl management has had little impact on the mammal fauna. However, results also demonstrate that knowledge of the local fauna remains incomplete. There is need for additional surveys employing methods to target poorly represented groups, as well as ongoing monitoring to assess the results of habitat restoration and anticipated changes to habitat and wildlife that may arise from shifting human land use and future climate change.

Acknowledgments

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Appendix 1

Specimen-based records of occurrence. Museum acronyms: BYU (Brigham Young University Life Science Museum), CSULB (California State University, Long Beach), DMNS (Denver Museum of Nature and Science), HSUVM (Cal Poly Humboldt Vertebrate Museum), LSUMZ (Louisiana State University Museum of Zoology), MSB (Museum of Southwestern Biology, University of New Mexico), MVZ (Museum of Vertebrate Zoology, University of California, Berkeley), UMNH (Natural History Museum of Utah, University of Utah), UMZM (University of Montana Zoological Museum), UNR (University of Nevada, Reno), UWBM (Burke Museum of Natural History and Culture, University of Washington), UWYMV (University of Wyoming Museum of Vertebrates).

Notiosorex crawfordi (*n* = 4). Utah: Tooele Co.: Dugway Proving Ground, site 18 C (BYU:Mamm:33205), Granite Mountain (BYU:Mamm:34204), Wig Mountain (BYU:Mamm:33207), Sapphire Mountain (BYU:Mamm:33208).

Sorex merriami (*n* = 5). Utah: Juab Co.: 10 miles south of Eureka (MSB:Mamm:102730). Tooele Co.: 6 miles south of Vernon (MSB:Mamm:102731). Nevada: Elko Co.: Adobe Hills (UMNH:Mamm:33810). White Pine Co.: 2 mi S Baker (MVZ:Mamm:175243), Snake Range, Strawberry Canyon, Great Basin National Park (UMNH:Mamm:32696).

Sorex preblei (*n* = 4). Utah: Juab Co.: Fish Springs National Wildlife Refuge, vicinity North Spring (UMNH:Mamm:41343). Tooele Co.: Horseshoe Springs (BYU:Mamm:14711); Timpie Springs (UMNH:Mamm:28208). Nevada: Elko Co.: Ruby Valley USFS station (UMNH:Mamm:32405).

Sorex tenellus (*n* = 4). Utah: Juab Co.: Deep Creek Mountains, Granite Creek Canyon (UMNH:Mamm:37371). Nevada: Elko C.: south side of Lamoille Canyon (UMNH:Mamm:31717). White Pine Co.: Snake Range, Lehman Creek, 1.8 km E boundary of Great Basin National Park (UMNH:Mamm:33798), Bald Mountain (UMNH:Mamm:39817).

Sorex vagrans (n = 53). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14624, UMNH:Mamm:26367, 30582–39587, 30605–30609, 30613–30618, 30755, 30761, 30789, 35980–35990, 36020, 40039–40042, 40470–40474, 41344–41346), 3.6 km E, 3.5 km S summit Ibapah Peak, Deep Creek Range (BYU:Mamm:36737), Granite Creek Canyon (UMNH:Mamm:37372). Tooele Co.: Timpie Springs Waterfowl Management Area (UMNH:Mamm:30425), Skull Valley, 7 mi N Iosepa (UMNH:Mamm:26562). Nevada: Elko Co.: W side Ruby Lake, 3 mi N Elko County line (MVZ:Mamm:40126); White Pine Co.: Cleveland Ranch, Spring Valley (MVZ:Mamm:45889), Snake Range, mouth of Pole Canyon (UMNH:Mamm:38515).

Tadarida brasiliensis (*n* = 7). Utah: Juab Co.: 4 mi N Nephi, Neff Farm (CSULB:Mamm:252). Millard County, Holden (UMNH:Mamm:17214). Tooele Co.: Dugway (UMNH:Mamm:27271), 4 mi E north end of Camelback Mountain (UMNH:Mamm:27275), south end of Cedar Mountains (UMNH:Mamm:27274). Nevada: White Pine Co.: Baker (UMNH:Mamm:33801), Snake Range, Rose Guano Cave (UMNH:Mamm:42458).

Antrozous pallidus (*n* = 5). Utah: Juab Co.: Bagley Ranch, Callao (UMNH:Mamm:27248). Millard Co.: Desert Experimental Range Station (UWYMV:Mamm:6208). Tooele Co.: Dugway (UMNH:Mamm:27244), 2 mi NE Camelback Mountain (UMNH:Mamm:25769). Nevada: White Pine Co.: Rose Guano Cave, Snake Range (UMNH:Mamm:27245).

Corynorhinus townsendii (*n* = 9). Utah: Juab Co.: 12 mi W Fish Springs Wildlife Refuge (UMNH:Mamm:23958), Trout Creek Canyon, Deep Creek Mountains (UMNH:Mamm:27255). Millard Co.: Gandy Mountain Cave (UMNH:Mamm:13485). Tooele Co.: Dugway Mountains (UMNH:Mamm:27250), Little Granite Mountain (UMNH:Mamm:32781), Gold Hill (UMNH:Mamm:7213). Nevada: Elko Co.: 1 mi N Hwy. 40, 2 mi E Carlin (UNR 1184). White Pine Co.: Schell Creek Range, Piermont Canyon (UMNH:Mamm:42445), Cleveland Ranch (MVZ:Mamm:45899).

Eptesicus fuscus (*n* = 7). Utah: Juab Co.: Callao (UMNH:Mamm:27232), Cherry Creek (LSUMZ:Mamm:37219). Millard Co.: Oak City (UMNH:Mamm:27403). Tooele Co.: Simmonds Ranch, 2 mi E Ibapah (UMNH:Mamm:7219), Terra, 0.5 mi W Johnsons Pass (UMNH:Mamm:27233). Nevada: Elko Co.: Ruby Valley USFS Station (UMNH:Mamm:32395). White Pine Co.: Great Basin National Park Visitor Center, Baker (UMNH:Mamm:42457).

Lasionycteris noctivagans (*n* = 5). Utah, Juab Co.: Fish Springs (UMNH:Mamm:27260), 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU:Mamm:36739), Callao (UMNH:Mamm:27261). Millard Co.: Oak Creek Reservoir (UMNH:Mamm:14576). Tooele Co.: 2 mi NE north end of Camelback Mountain (UMNH:Mamm:27264).

Lasiurus cinereus (*n* = 3). Utah: Juab Co.: 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU:Mamm:36740). Tooele Co.: 3 mi N Iosepa (UMNH:Mamm:27249), English Village, Dugway Proving Grounds (BYU:Mamm:24971).

Myotis californicus (*n* = 3). Utah: Millard Co.: Muddy Spring, House Range (MSB:Mamm:273927). Tooele Co.: 1 mi E Ibapah (UMNH:Mamm:4424). Nevada: Elko Co.: Monarch Mine, Spruce Mountain (HSUVM:Mamm:6826).

Myotis ciliolabrum (*n* = 12). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14625; UMNH:Mamm:26356, 29438, 29439, 31916). Millard Co.: 4 mi E Oak City (UMNH:Mamm:18992). Tooele Co.: 1 mi E Ibapah (UMNH:Mamm:7217), Parrish Ranch, 5 mi N Ibapah (UMNH:Mamm:7214), 5 mi NW Camelback Mountain (UMNH:Mamm:26354), south end of Cedar Mountains (UMNH:Mamm:26366). Nevada: Elko Co.: W side Ruby Lake, 3 mi N county line (MVZ:Mamm:46860). White Pine Co.: south side Mt. Moriah (MVZ:Mamm:45900).

Myotis evotis (*n* = 6). Utah: Juab Co.: Cherry Creek (LSUMZ:Mamm:37217). Millard Co.: Oak Creek Canyon (UMNH:Mamm:13791). Tooele Co.: Harker Canyon (BYU:Mamm:18086), southwest base of Stansbury Mountains (UMNH:Mamm:27242). Nevada: Elko Co.: Monarch Mine, Spruce Mountain (HSUVM:Mamm:6827). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78382).

Myotis lucifugus (*n* = 7). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:43280, 43281), 4.5 km E, 5.4 km S summit Ibapah Peak, Deep Creek Range (BYU:Mamm:36741), York, 5 mi S Santaquin (UMNH:Mamm:7223). Millard Co.: Pavant Range, Robins Valley (UMNH:Mamm:17128). Tooele Co.: Skull Valley (UMNH:Mamm:27235). Nevada: Elko Co.: W side Ruby Lake, 3 mi N county line (MVZ:Mamm:46861).

Myotis thysanodes (n = 5). Utah: Utah Co.: N Fork Provo Canyon (BYU:Mamm:10439). Garfield Co.: 1.5 mi N Pink Cliffs Motel (MSB:Mamm:115748). Washington Co.: Pine Valley, 36 mi N St. George (UMNH:Mamm:14658). Wayne Co.: Ackland Spring, Capitol Reef National Park (MSB:Mamm:116747). Nevada: Clark Co.: Moapa Valley National Wildlife Refuge (MSB:Mamm:286720), 10 mi southeast of Riverside (CSULB:Mamm:7201).

Myotis volans (n = 7). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14626), Deep Creek Mountains, Basin Creek Pass (UMNH:Mamm:7228), Cherry Creek (LSUMV:Mamm:37218). Tooele Co.; south end of Cedar Mountains (UMNH:Mamm:27237), Hard to Beat Canyon, Sheeprock Mountains (BYU:Mamm:18598). Nevada: Elko Co.; Monarch Mine, Spruce Mountain (HSUVM:Mamm:6830). White Pine Co.; 7.5 mi SE Mt. Moriah (MVZ:Mamm:78368).

Myotis yumanensis (*n* = 7). Utah: Cache Co.: Logan (BYU:Mamm:13041). Emery Co.: Huntington Canyon (BYU:Mamm:18135). Iron Co.: Cedar City (UMNH:Mamm:44409). Utah Co.: Provo (BYU:Mamm:41155). Washington Co.: Manganese Wash, Santa Clara River (UMNH:Mamm:45827). Nevada: Clark Co.: Moapa National Wildlife Refuge (MSB:Mamm:286738). Idaho: Owyhee Co.: Owyhee River, 12 mi N Nevada line (MVZ:Mamm:38341).

Parastrellus hesperus (*n* = 7). Utah, Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:13156, 14627, 14628; UMNH:Mamm:30826). Tooele Co.: Staley Spring, NW edge of Dugway Range (UMNH:Mamm:29435), old Lincoln Highway, 18 mi SW Orr's Ranch, Skull Valley (UMNH:Mamm:3530), 3 mi E north end of Camelback Mountain (UMNH:Mamm:27268).

Lynx rufus (n = 18). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15625–15632), Trout Creek (UMNH:Mamm:37908), 7 mi W Joy (UMNH:Mamm:10930). Millard Co.: 4 mi S Gandy (MVZ:Mamm:78412), 30 mi W Delta (BYU:Mamm:2274). Tooele Co.: Gold Hill (UMNH:Mamm:7105), Dugway Valley (UMNH:Mamm:14027), Simpson Mountains (UMNH:Mamm:14028). Nevada: Elko Co.: E slope Spruce Valley Mt (MVZ:Mamm:120838). White Pine Co.: Snake Range, Great Basin National Park, Lower Lehman campground (UMNH:Mamm:42259), Duck Creek (MSB:Mamm:101530).

Puma concolor (*n* = 7). Utah: Juab Co.: Deep Creek Mountains, Granite Creek Canyon (UMNH:Mamm:5466), mouth of Red Cedar Canyon, east side Deep Creek Mountains (UMNH:Mamm:25319). Millard Co.: Eightmile Creek (UMNH:Mamm:14971). Nevada: Elko Co.: Cold Creek, Ruby Mountains (MVZ:Mamm:97013). White Pine Co.: Chokecherry Creek, Snake Range (MVZ:Mamm:88066), Great Basin National Park, near Baker Creek (UMNH:Mamm:42261), Great Basin National Park, Big Wash (UMNH:Mamm:42260).

Canis latrans (*n* = 10). Utah, Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15616, 15617), Cane Springs (UMNH:Mamm:3978), 7 mi W Joy (UMNH:Mamm:10923), Trout Creek (UMNH:Mamm:37889). Millard Co.: 6 mi W Deseret (MVZ:Mamm:80806), vicinity of Garrison (MVZ:Mamm:78406). Tooele Co.: Grassy Mountains (UMNH:Mamm:8798). Nevada: Elko Co.: 5 mi W Wendover (UMNH:Mamm:8797). White Pine County, Steptoe Creek (MVZ:Mamm:454906).

Urocyon cinereoargenteus (n = 5). Utah: Juab Co.: between Callao and Trout Creek (UMNH:Mamm:25326). Tooele Co.: Clifton Flat, 5 mi S Gold Hill (UMNH:Mamm:10950). Nevada: White Pine Co.: mouth of Snake Creek (MVZ:Mamm:47145), Lexington Creek, Snake Range (MVZ:Mamm:129389). Snake Range, 0.4 mi N Stella Lake, Great Basin National Park (UMNH:Mamm:31223).

Vulpes macrotis (n = 16). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15264, 15620–15624), Topaz Mountain (UMNH:Mamm:7142), 1 mi SW Callao (UMNH:Mamm:29090). Millard Co.: Swasey Reservoir (BYU:Mamm:3850), 28 mi E NV border, US 50 (BYU:Mamm:11099), Desert Experimental Range Station (UMNH:Mamm:24152). Tooele Co.: Dugway Valley (UMNH:Mamm:14100), 5 mi NE north end of Granite Mountain (UMNH:Mamm:29068), 2 mi S Simpson Springs (UMNH:Mamm:10949). Nevada: Elko Co.: 3 mi E Silverzone Pass (UMNH:Mamm:30442); White Pine Co.: near Ely (BYU:Mamm:4229).

Vulpes vulpes (*n* = 1). Utah: Millard Co.: Garrison (UMNH:Mamm:42254).

Neogale frenata (*n* = 10). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15251), 3 mi N Nephi (UMNH:Mamm:28891). Millard Co.: Deseret (MVZ:Mamm:80798), Pavant Range, Robins Valley (UMNH:Mamm:15612). Tooele Co.: west base of Little Granite Mountain (UMNH:Mamm:27191), 4 mi N Wig Mountain (UMNH:Mamm:27202), south end Cedar Mountains (UMNH:Mamm:27198). Nevada: Elko Co.: East Humboldt Range, 0.35 km N, 0.30 km W Jerry Crab Spring (UMNH:Mamm:32113). White Pine Co.: Baker Creek (MVZ:Mamm:41504), Schell Creek Range, Steptoe Creek (MSB:Mamm:227148).

Taxidea taxus (*n* = 12). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15618, 15619), 2 mi W Trout Creek (UMNH:Mamm:2313). Millard Co.: Conger Springs (BYU:Mamm:11662), 4 mi W Deseret (MVZ:Mamm:80801). Tooele Co.: Ibapah (UMNH:Mamm:3625), 8 mi SW Simpson Spring, Table Mountain (UMNH:Mamm:12487), Wig Mountain, west side of Cedar Mountains (UMNH:Mamm:25321). Nevada: Elko Co.: Montello (MVZ:Mamm:24579), 25 mi S Wells (UMZM:Mamm:13949). White Pine Co.: 7 mi W Utah state line (UMNH:Mamm:7614), Lehman Creek (MVZ:Mamm:41499).

Mephitis mephitis (*n* = 10). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15267, 15634, 25317, 25318). Millard Co.: 1 mi S Deseret (UMNH:Mamm:25371), Black Rock (UMNH:Mamm:17046). Tooele Co.: 0.5 mi N north end of Granite Mountain (UMNH:Mamm:25373), 4 mi N north end of Camelback Mountain (UMNH:Mamm:25372). Nevada: Elko Co.: Mary's River, 26 mi N Deeth (MVZ:Mamm:67774). White Pine Co.: Home Farm, 2.4 km N, 5.2 km W Baker (UMNH:Mamm:41184).

Spilogale gracilis (*n* = 12). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:15252, 15258, 15421), Callao (BYU:Mamm:4568), Deep Creek Range, Granite Creek Canyon, 3.66 km S, 3.45 km E summit Ibapah Peak (UMNH:Mamm:37576), Deep Creek Mountains, above junction of Birch and Trout creeks (UMNH:Mamm:7616). Millard Co.: Confusion Range (UMNH:Mamm:27185), 16 mi N Deseret (MVZ:Mamm:80799). Tooele Co.: Granite Peak (BYU:Mamm:11613), Dugway Valley (UMNH:Mamm:27184). Nevada: Elko Co.: Ruby Mountains, vicinity of Flyn Spring (UMNH:Mamm:31868). White Pine Co.: McGill, Gallogher's Gap (UMZM:Mamm:12280).

Bassariscus astutus (*n* = 5). Utah: Juab Co.: Fish Springs National Wildlife Refuge (UMNH:Mamm:27187), Deep Creek Mountains, Birch Creek Canyon (UMNH:Mamm:5588), mouth of Granite Creek Canyon (UMNH:Mamm:24090), mouth of Red Cedar Canyon (UMNH:Mamm:27188). Nevada: White Pine Co.: Baker (UMNH:Mamm:42255).

Procyon lotor (*n* = 1). Utah: Tooele Co.: Oquirrh Mountains, Middle Canyon (UMNH:Mamm:28778).

Antilocapra americana (*n* = 4). Utah: Millard Co.: Desert Experimental Range Station (UMNH:Mamm:23431). Tooele Co.: 8 mi SW Simpson Spring, Table Mountain (UMNH:Mamm:12486), 5 mi N Camelback Mountain (UMNH:Mamm:14645), Dugway Valley (UMNH:Mamm:24927).

Odocoileus hemionus (*n* = 8). Utah: Juab Co.: 5 mi S Nephi (BYU:Mamm:6658). Millard Co.: 2 mi N Oak City (BYU:Mamm:13331). Tooele Co.: Candy Creek, Cedar Mountains (UMNH:Mamm:24930), Benmore Guard Station (UMNH:Mamm:22377). Nevada: Elko Co.: Ruby Mountains, Lamoille Canyon (UMNH:Mamm:36531). White Pine Co.: Lehman Creek (MVZ:Mamm:42085), Baker Creek (MVZ:Mamm:42086), Duck Creek Valley (MSB:Mamm:103570).

Ovis canadensis (n = 5). Utah: Tooele Co.: west base of Granite Mountain (UMNH:Mamm:24944), west side of Lakeside Mountains (UMNH:Mamm:24941). Nevada: White Pine Co.: Hendry Creek, 1.5 mi E Mt. Moriah (MVZ:Mamm:79610), head of Baker Creek (MVZ:Mamm:88136), 0.25 mi SW Stella Lake (MVZ:Mamm:88137).

Ammospermophilus leucurus (*n* = 40). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14632–14639, 37079; UMNH:Mamm:3607, 8180, 23320, 24713, 25249, 30623–30625, 30627, 35873–35877, 35991–35994, 40467), Fish Springs Range, 1.5 mi W Pony Express monument (UMNH:Mamm:43282), 0.05 km N, 0.5 km W junction Snake Valley and Granite Creek roads (UMNH:Mamm:37175), 5.5 mi S, 7.8 mi E Callao (MVZ:Mamm:149696), 6.7 mi S, 5.3 mi E Callao (MSB:Mamm:36078), 1.5 mi N Topaz Mountain (UMNH:Mamm:27799). Millard Co.: Tule Valley (UWYMV:Mamm:6070), Marjum Pass, House Range (UMNH:Mamm:18403), Gandy Salt Marsh (BYU:Mamm:15061). Tooele Co.: Gold Hill (BYU:Mamm:3569), Little Granite Mountain (UMNH:Mamm:25246), north end of Camelback Mountain (UMNH:Mamm:25258). Nevada: White Pine Co.: 1 mi N Baker (UMNH:Mamm:38165).

Marmota flaviventris (*n* = 5). Utah: Juab Co.: 4 mi W Eureka, 1 mi E Elberta (UMNH:Mamm:28561). Tooele Co.: 9 mi S Vernon, 1 mi E Benmore (BYU:Mamm:5379), Stansbury Mountains, Deseret Peak (UMNH:Mamm:25514). Nevada: Elko Co.: Lamoille Creek, Ruby Mts (MVZ:Mamm:120755). White Pine Co.: Snake Range, Great Basin National Park, Baker Creek Canyon (UMNH:Mamm:41373).

Tamias dorsalis (*n* = 9). Utah: Juab Co.: 4.0 km E, 4.4 km S summit Ibapah Peak, Deep Creek Range (BYU:Mamm:36760), mouth of Indian Farm Canyon, 7 mi SW Callao (UMNH:Mamm:25706), Cherry Creek, 7 mi W Eureka (UMNH:Mamm:7881). Millard Co., 1 mi N Sinbad Spring, House Range (UMNH:Mamm:29061). Tooele Co.: 1.5 mi N Gold Hill (UMNH:Mamm:26730), east slope Granite Peak (UMNH:Mamm:26431), Indian Springs, Simpson Mountains (UMNH:Mamm:14119). Nevada: Elko Co.: Ferguson Hills (UMNH:Mamm:35289). White Pine Co.: Smith Creek, Mt. Moriah (MVZ:Mamm:78624).

Tamias minimus (*n* = 14). Utah: Juab Co.: Deep Creek Mountains, Granite Creek Canyon (UMNH:Mamm:26778), mouth of Indian Farm Canyon (UMNH:Mamm:26671). Millard Co.: 4 mi S Gandy (MVZ:Mamm:78571), Pavant Range (UMNH:Mamm:17531). Tooele Co.: Clifton Flat, 5 mi S Gold Hill (UMNH:Mamm:26667), NW of Granite Peak (UMNH:Mamm:26423), 8 mi SW Wig Mountain (UMNH:Mamm:26765), 3 mi W Camelback Mountain (UMNH:Mamm:25957). Nevada: Elko Co.: Dolly Varden Mountains, Victoria Mine (UMNH:Mamm:37291), Cherry Creek Mountains, Corral Canyon (DMNS:Mamm:11690), Monarch Mine, Spruce Mountain (HSUVM:Mamm:6741). White Pine Co.: E side Shellbourne Pass (MVZ:Mamm:45971), 5 mi SE Greens Ranch, Steptoe Valley (MVZ:Mamm:78569), Snake Range, Strawberry Creek Canyon, Great Basin National Park (UMNH:Mamm:32701).

Urocitellus mollis (*n* = 12). Utah: Juab Co.: 5 mi SE Fish Springs Refuge (UMNH:Mamm:24851), 0.7 mi N Black Rock Hills (UMNH:Mamm:24937), north end of Thomas Range (UMNH:Mamm:7860), 2.5 mi E Topaz Mountain (UMNH:Mamm:27839). Millard Co.: Deseret (MVZ:Mamm:80808), Mormon Gap, 15 mi S, 6 mi E Garrison (UMNH:Mamm:28054). Tooele Co.: 2 mi S Ibapah (UMNH:Mamm:27662), 5.7 mi W Simpson Springs (UMNH:Mamm:27922). Nevada: Elko Co.: Currie (UWBM:Mamm:42741), Montello (MSB:Mamm:291916). White Pine Co.: 9 mi S Schellbourne Pass, Steptoe Valley (MVZ:Mamm:45937), 7 mi SW Osceola, Spring Valley (MVZ:Mamm:41530).

Otospermophilus variegatus (*n* = 10). Utah: Juab Co.: 7 mi S Callao (UWBM:Mamm:42860), Granite Creek Canyon, Deep Creek Mountains (UMNH:Mamm:25432), Ferner Valley, 10 mi E Nephi (MSB:Mamm:102744). Millard Co.: Oak Creek Canyon, 2 mi E Oak City (UMNH:Mamm:21935), 0.5 mi above Meadow (UMNH:Mamm:24573). Tooele Co.: Indian Springs, west side of Simpson Mountains (UMNH:Mamm:13575), Little Valley Ranger Station, Sheeprock Mountains (UMNH:Mamm:8229). Nevada: White Pine Co.: Cherry Creek (MVZ:Mamm:45931), Cleve Creek, Schell Creek Range (MVZ:Mamm:45927), Snake Creek, 3 mi below Johnson Lake, Snake Range (UNR:Mamm:196).

Dipodomys microps (*n* = 67). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:2900–2902, 14642–14657; UMNH:Mamm:3585, 3603, 3604, 24157, 24158, 24178, 24211, 30577, 30596–30598, 30620, 30621, 30629, 30630, 30631, 30756, 30766, 30797, 30801, 35879, 35996–36004, 42512, 42521–42525), south side of Topaz Mountain (CSULB:Mamm:8657), 0.25 km E, 0.20 km S junction Granite Creek Road and Snake Valley Road (BYU:Mamm:36811), Joy (BYU:Mamm:2736). Millard Co.: Tule Valley (UWYMV:Mamm:501), 1 mi SE Gandy (MVZ:Mamm:78992). Tooele Co.: Dugway Proving Ground (MSB:Mamm:61909), Dugway Valley (UWBM:Mamm:45895), 3 mi SW Gold Hill (UMNH:Mamm:25221). Nevada: Elko Co.: 3.8 km S, 2.8 km W Leppy Peak (UMNH:Mamm:45602). White Pine Co.: 5 mi SE Greens Ranch, Steptoe Valley (MVZ:Mamm:78965), Spring Valley (MSB:Mamm:286841), 1 mi N Baker (UMNH:Mamm:38169).

Dipodomys ordii (*n* =27). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14658–14660; UMNH:Mamm:3583, 3584, 3601, 3602, 3622, 30776, 30777, 30781, 36005, 42526), Sand Pass (UMNH:Mamm:25176), Callao (BYU:Mamm:3237), south side of Topaz Mountain (CSULB:Mamm:8655), Joy (BYU:Mamm:2730), Trout Creek (MVZ:Mamm:85175). Millard Co.: Tule Valley (UWYMV:Mamm:495), Gandy Salt Marsh (BYU:Mamm:15079). Tooele Co.: Dugway Proving Grounds (MSB:Mamm:61914), 1 mi W Five Mile Hill summit (UMNH:Mamm:25206). Nevada: Elko Co.: Salt Springs (MVZ:Mamm:46148), 3 mi S Shanty Town, Ruby Lake Wildlife Refuge (UMNH:Mamm:29319). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78916), Spring Valley (MSB:Mamm:286845), 1 mi N Baker (UMNH:Mamm:38181).

Microdipodops megacephalus (*n* = 15). Utah: Juab Co.: 3 mi SSW Fish Springs Refuge (UMNH:Mamm:24772), 5.5 mi S, 7.8 mi E Callao (MSB:Mamm:35602), 6.7 mi S, 5.3 mi E Callao (MSB:Mamm:35597), 7.7 mi N, 2.7 mi E Callao (MVZ:Mamm:159898), Maxfield Ranch, 4.5 mi NE Trout Creek (UMNH:Mamm:25849), 4 mi SW Trout Creek (UMNH:Mamm:25847). Millard Co.: Tule Valley (UWYMV:Mamm:447), 5 mi S Gandy (MVZ:Mamm:79083). Tooele Co.: 8 mi E north end of Granite Mountain (UMNH:Mamm:13553), 3 mi N Granite Mountain (UMNH:Mamm:25413), 2 mi NE north end of Camelback Mountain (UMNH:Mamm:25428). Nevada: Elko Co.: 2 mi SW Cobre (MVZ:Mamm:47623), east end of Brown Dike, Ruby Lake National Wildlife Refuge (UMNH:Mamm:35295). White Pine Co.: 7 mi SW Osceola, Spring Valley (MVZ:Mamm:41823), Spring Valley, 4 mi S Shoshone (MVZ:Mamm:59513).

Chaetodipus formosus (*n* = 38). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:2876–2879, 14661–14668, 32734; UMNH:Mamm:30579, 30628, 30642, 30643, 30752, 30767, 30768, 30807, 35878, 35995, 43284, 43288, 43289), 3 mi W Callao (UMNH:Mamm:25339), junction Granite Creek and Snake Valley roads (UMNH:Mamm:37184), Sand Pass (UMNH:Mamm:25029), south side Topaz Mountain (CSULB:Mamm:8687). Millard Co.: Swasey Springs, House Range (UMNH:Mamm:25380), Gandy Salt Marsh (BYU:Mamm:15082), Black Rock (UMNH:Mamm:28243). Tooele Co.: 3.0 km E, 0.4 km S Young Peak, Deep Creek Range (BYU:Mamm:40203), NE base of Dugway Mountains (UMNH:Mamm:25013). Nevada: Elko Co.: E slope Goshute Mts (MVZ:Mamm:197195), 3.8 km S, 2.8 km W Leppy Peak (UMNH:Mamm:45605). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78854).

Perognathus longimembris (*n* = 15). Utah: Juab Co.: 1 mi S Callao (UMNH:Mamm:25773), 0.25 km E, 0.20 km S junction Granite Creek Road and Snake Valley Road (BYU:Mamm:36777), Bobcat Ranch, above confluence of Birch and Trout creeks (UMNH:Mamm:7523), Leland Harris Spring (BYU:Mamm:15083), Sand Pass (UMNH:Mamm:25779), Joy (BYU:Mamm:2743). Millard Co.: Tule Valley (UWYMV:Mamm:6015), Desert Experimental Range (MSB:Mamm:103880). Tooele Co.: NW base of Table Mountain (UMNH:Mamm:25787), 4 mi NW north end Camelback Mountain (UMNH:Mamm:25974), 6 mi NE north end Granite Mountain (UMNH:Mamm:25786). Nevada: Elko Co.: Pilot Ranch, 16 mi S Montello (UMNH:Mamm:13551), Ruby Lake National Wildlife Refuge (UMNH:Mamm:36121). White Pine Co.: 2 mi E Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78767), 1.6 mi N Baker (MVZ:Mamm:225200).

Perognathus mollipilosus (*n* = 15). Utah: Juab Co.: Callao (BYU:Mamm:3099), Deep Creek Mountains, Granite Creek Canyon (UMNH:Mamm:24993), Joy (BYU:Mamm:2767). Millard Co.: House Range, 2.5 km N, 4.5 km E Swasey Peak (UMNH:Mamm:45814), N side Marjum Pass, House Range (BYU:Mamm:7391), 7 mi SW Skull Rock Pass (MVZ:Mamm:179595). Tooele Co.: north end Dugway Mountains (UMNH:Mamm:23338), Simpson Mountains, upper Judd Creek Canyon

(UMNH:Mamm:25071), Clifton Flat (UMNH:Mamm:6360). Nevada: Elko County, Dolly Varden Mountains, 0.3 km S, 0.1 km W Victoria Mine (UMNH:Mamm:37292), E slope Spruce Mountain (MVZ:Mamm:120787), East Humboldt Range. 0.15 km E Davis Spring (UMNH:Mamm:32091). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78847), east side Schellbourne Pass (MVZ:Mamm:46109), Spring Valley, 3 km N, 4 km W Osceola (UMNH:Mamm:40939).

Thomomys bottae (*n* = 43). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:2856, 14640, 14641, UMNH:Mamm:3573–3582, 3615, 25762–25764, 26613, 26614, 40475–40479, 41347–41349, 42503–42505), Sand Pass (UMNH:Mamm:27332), Callao (UMNH:Mamm:14451), Trout Creek (UMNH:Mamm:1941). Millard Co.: Tule Valley (UWYMV:Mamm:6005), Swasey Spring, House Range (UMNH:Mamm:2524), Marjum Pass, House Range (UMNH:Mamm:26594). Tooele Co.: north end of Dugway Range (UMNH:Mamm:27333), 1 mi W Five Mile Hill summit, Dugway Valley (UMNH:Mamm:14443), north end of Granite Mountain (UMNH:Mamm:11159). Nevada: Elko County, Pilot Ranch, 16 mi S Montello (UMNH:Mamm:14367). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:78660), 2.5 mi E Baker (MVZ:Mamm:41679), Snake Range, Snake Creek Canyon, Shoshone Campground (UMNH:Mamm:30531).

Lemmiscus curtatus (*n* = 12). Utah: Juab Co.: Deep Creek Mountains, mouth of Indian Farm Canyon, 7 mi SW Callao (UMNH:Mamm:25564), Queen of Sheba Canyon (UMNH:Mamm:3731), 2.7 km N, 1.3 km E Indian Springs (UMNH:Mamm:31502). Millard Co.: north side Marjum Pass, House Range (BYU:Mamm:7393). Tooele Co.: Clifton Flat, 7 mi SW Gold Hill (UMNH:Mamm:4588), 0.5 mi E Simpson Springs (UMNH:Mamm:30125). Nevada: Elko Co.: Dolly Varden Mountains, 1.1 km S, 0.5 km W Victoria Mine (UMNH:Mamm:37321), Cobre (MVZ:Mamm:68602), HD Summit, 20 mi N Wells (UMNH:Mamm:28616). White Pine Co.: Hendry Creek, Mt. Moriah (MVZ:Mamm:79544), Cleve Creek, Schell Creek Range (MVZ:Mamm:46374), 2 mi NW summit of Sacramento Pass (UMNH:Mamm:26859).

Microtus longicaudus (*n* = 15). Utah: Juab Co.: Deep Creek Mountains, Indian Farm Canyon (UMNH:Mamm:26856), Tom's Creek, Deep Creek Mountains (HSUVM:Mamm:6536), Deep Creek Mountains, Granite Creek Canyon (UMNH:Mamm:26833). Millard Co.: Sinbad Spring, House Range (UMNH:Mamm:29062), 4 mi E Oak City (UMNH:Mamm:23854), Filmore (BYU:Mamm:12434). Tooele Co.: 0.5 mi E Simpson Springs (UMNH:Mamm:30124), Indian Springs, west side Simpson Mountains (UMNH:Mamm:26628), Hard to Beat Canyon (BYU:Mamm:18995). Nevada: Elko Co.: 12.5 mi SW Wendover (UMNH:Mamm:26648), Debbs Creek, Pilot Range (BYU:Mamm:33868), E slope Spruce Mountain (MVZ:Mamm:120802). White Pine Co.: Smith Creek, Mt. Moriah (MVZ:Mamm:71590), Snake Range, mouth of Pole Canyon (UMNH:Mamm:38404), Snake Range, south fork Big Wash, Great Basin National Park (UMNH:Mamm:30726).

Microtus montanus (n = 65). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14669, 14670; UMNH:Mamm:3596, 3597, 3605, 3606, 25565–25569, 26632, 26633, 26824, 27180, 27183, 27225, 30588–30591, 30611, 30612, 30769, 30770, 30782, 30805, 35880–35887, 40051–40053, 40480–40093, 41361–41363), Bagley Ranch, Callao (UMNH:Mamm:25570). Millard Co.: 5 mi S Garrison (MVZ:Mamm:79465). Tooele Co.: 1 mi E Ibapah (UMNH:Mamm:7461), 7.5 mi E north tip of Granite Mountain (UMNH:Mamm:25702). Nevada: Elko Co.: Ferguson Springs (UMNH:Mamm:35329), Salt Spring (MVZ:Mamm:46373), Currie (UWBM:Mamm:63810). White Pine Co.: 1 mi N Baker (UMNH:Mamm:38182), Cleveland Ranch, Spring Valley (MVZ:Mamm:46275), 7 mi SW Osceola. Spring Valley (MVZ:Mamm:42060).

Ondatra zibethicus (*n* = 95). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:396–399, 14678–14680, 34588–34602; UMNH:Mamm:15246–15250, 15259–15263, 15268–15272, 15417–15420, 15496–15505, 15580–15602, 15605–15609, 17507–17510, 40468, 40469, 41364–41366), 1.25 mi SW Mills (UMNH:Mamm:24097). Millard Co.: 10 mi N Deseret (MVZ:Mamm:80810), 5 mi S Garrison (MVZ:Mamm:79570). Tooele Co.: Ibapah (UMNH:Mamm:9705), Horseshoe Springs (BYU:Mamm:14731). Nevada: Elko Co.: west side of Ruby Lake, 3 mi N Elko County line (MVZ:Mamm:40132). White Pine Co.: Snake Range, Lehman Creek, east of Great Basin National Park (UMNH:Mamm:33774).

Neotoma lepida (*n* = 42). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:2860, 14676, 14677, 37091–37098; UMNH:Mamm:3598, 30638–30641, 35899, 35900, 36008–36014, 40049, 40050), Deep Creek Range, Granite Creek Canyon (UMNH:Mamm:37215), Trout Creek (BYU:Mamm:12859), south side of Topaz Mountain (CSULB:Mamm:8670). Millard Co., Tule Valley (UWYMV:Mamm:741), Swasey Spring (UMNH:Mamm:2514), Gandy Salt Marsh (BYU:Mamm:15096). Tooele Co.: Dugway Proving Ground, Cane Spring (MSB:Mamm:86623), Granite Peak (UMNH:Mamm:26288), Dugway Valley (UWBM:Mamm:61464). Nevada: Elko Co.: Ferguson Hills (UMNH:Mamm:35302). 9.8 km S, 4.4 km W West Wendover (UMNH:Mamm:45583), 1.5 mi SW Currie (MVZ:Mamm:134418). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:79383), South Snake Ridge (UNR:Mamm:4092), 1 mi N Baker (MVZ:Mamm:42021).

Onychomys leucogaster (*n* = 17). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14681; UMNH:Mamm:30578), 7 mi S Fish Springs (UMNH:Mamm:3616), Trout Creek UMNH:Mamm:1952), Little Sahara near Cherry Creek Wash (BYU:Mamm:24538). Millard Co.: Tule Valley (UWYMV:Mamm:3773), 4 mi S Gandy (MVZ:Mamm:79117), Fishlake National Forest, 7 km S Oak City (MSB:Mamm:291859). Tooele Co.: 2 mi N Fish Springs Mountains (UMNH:Mamm:25465), Dugway Valley (UWBM:Mamm:63400), 3 mi SW Gold Hill (UMNH:Mamm:24908). Nevada: Elko Co.: Tecoma (MVZ:Mamm:68245), Cobre (MVZ:Mamm:68280), East Humboldt Range, 0.15 km E Davis Spring (UMNH:Mamm:32112). White Pine Co.: 0.5 km N Baker (UMNH:Mamm:30815), Spring Valley (MSB:Mamm:286820), Steptoe Creek. 5.5 mi SE Ely (MVZ:Mamm:46160).

Peromyscus crinitus (*n* = 46). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14682–14691; UMNH:Mamm:30580, 30581, 30644–30648, 35901–35912, 36015–36017), 7 mi S Fish Springs (UMNH:Mamm:3617), mouth of Granite Creek Canyon, Deep Creek Mountains (UMNH:Mamm:26305). Deep Creek Range, confluence of Birch and Trout creeks (UMNH:Mamm:9869). Millard Co.: Gandy Salt Marsh (BYU:Mamm:15097), Marjum Pass, House Range (UMNH:Mamm:26141), west of Meadow (UMNH:Mamm:28503). Tooele Co.: north end of Dugway Mountains (UMNH:Mamm:26314), NE side of Granite Peak Range (UMNH:Mamm:25915), 1 mi SW Gold Hill (UMNH:Mamm:25981). Nevada: Elko Co.: 3.8 km S, 2.8 km W Leppy Peak (UMNH:Mamm:45582), 9.8 km S, 4.4 km W West Wendover (UMNH:Mamm:45613). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:79175), Cleve Creek, Schell Creek Range (MVZ:Mamm:46174), Cherry Creek (MVZ:Mamm:46238).

Peromyscus maniculatus (*n* = 132). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14694–14697; UMNH:Mamm:3586–3595, 3608–3614, 26914, 26961, 27012, 27065, 30595, 30601–30604, 30636, 30637, 30751, 30757–30759, 30764, 30765, 30774, 30775, 30783, 30796, 30798, 30800, 30802–30804, 30806, 35913–35948, 36018, 40047, 40494–40496, 41353–41360, 42480–42489, 42510, 42511, 42513–42519), Fish Springs Range, 1.1 km N, 2.2 km W headquarters Fish Springs Refuge (UMNH:Mamm:40048), Sand Pass (UMNH:Mamm:26149), Trout Creek (BYU:Mamm:12275). Millard Co.: 4 mi S Gandy (MVZ: Mamm:79348), Swasey Spring, House Mountains (UMNH:Mamm:2517), 7 mi SW Skull Rock Pass (MVZ:Mamm:179599). Tooele Co.: Clifton Flat, 5 mi S Gold Hill (UMNH:Mamm:26173), old Lincoln Highway, 18 mi SW Orr's Ranch (UMNH:Mamm:3572), Simpson Springs (MSB:Mamm:76823). Nevada: Elko Co.: 3.8 km S, 2.8 km W Leppy Peak (UMNH:Mamm:45572), Dolly Varden Mountains, 1.1 km S, 0.5 km W Victoria Mine (UMNH:Mamm:37294), east side Spruce Mountain (MVZ:Mamm:120794). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:79309), 1 mi N Baker (UMNH:Mamm:38236), Snake Range, Great Basin National Park, 1.2 km N Pyramid Peak (UMNH:Mamm:41209).

Peromyscus truei (*n* = 15). Utah: Juab Co.: Fish Springs Range (BYU:Mamm:14698). Indian Farm Canyon, Deep Creek Range (BYU:Mamm:7211), confluence of Birch and Trout creeks, Deep Creek Mountains (UMNH:Mamm:10823). Millard Co.: N side Marjum Pass, House Range (BYU:Mamm:7394), 4 mi S Oak City (BYU:Mamm:34961), Desert Experimental Range Station (UMNH:Mamm:3843). Tooele Co.: 7 mi NE of north end Granite Peak Range (UMNH:Mamm:25901), Dugway Proving Ground, Cane Spring (MSB:Mamm:86661), 0.5 mi E Simpson Springs Pony Express site (UMNH:Mamm:30127). Nevada: Elko Co.: Ferguson Hills (UMNH:Mamm:35309), 0.70 km S, 2.15 km W Toano Peak (BYU:Mamm:35720), 0.5 mi W Debbs Creek, Pilot Peak (MVZ:Mamm:68468). White Pine Co.: Snake Creek Canyon, Great Basin NP (UNR:Mamm:4112), Cleve Creek, Schell Creek Range (MVZ:Mamm:46222), Cherry Creek (MVZ:Mamm:46225).

Reithrodontomys megalotis (*n* = 91). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14699–14708; UMNH:Mamm:3600, 26100, 30592–30594, 30599, 30600, 30622, 30632–30635, 30753, 30754, 30760, 30773, 30788, 30790, 30799, 35949–35979, 36019, 40043–40046, 40497–40499, 41350–41352, 42506–42509, 42520), Sand Pass (UMNH:Mamm:25801), 0.5 mi S Callao (UMNH:Mamm:25536), Deep Creek Range, Granite Creek Canyon, 5.6 km S, 4.85 km E Ibapah Peak (UMNH:Mamm:37550). Millard Co.: Tule Valley (UWYMV:Mamm:524), 1 mi SE Gandy (MVZ:Mamm:79142), Robinson Ranch, 17 mi S Gandy (MVZ:Mamm:79146). Tooele Co.: Clifton Flat, 3 mi SW Gold Hill (UMNH:Mamm:25528), 7 mi NE north end Granite Peak Range (UMNH:Mamm:25531), Indian Springs, Simpson Mountains (UMNH:Mamm:25802). Nevada, Elko Co.: Salt Springs (MVZ:Mamm:46164), Debbs Creek (BYU:Mamm:33880), 1.2 km S, 2.1 km W Toano Peak (BYU:Mamm:35727). White Pine Co.: 2 mi W Smith Creek Cave, Mt. Moriah (MVZ:Mamm:79139), 1 mi N Baker (MVZ:Mamm:41862), Sacramento Pass (UMNH:Mamm:25668).

Mus musculus (*n* = 43). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14671–14675; UMNH:Mamm:30610, 30619, 30626, 30762, 35888–35898, 36006, 36007, 30771, 30772, 30784–30787, 30791, 30792, 30778–30780, 30793–30795, Eureka (BYU:Mamm:34630), 4 mi W Mona (UMNH:Mamm:10527). Tooele Co.: Dugway Proving Ground (UMNH:Mamm:26338), Parrish Ranch, 5 mi N Ibapah (UMNH:Mamm:6331). Nevada, Elko Co.: 3 mi N Jarbidge (UNR:Mamm:2605). White Pine Co.: Baker (MVZ:Mamm:41874), 5.5 mi SE Ely (MVZ:Mamm:46274).

Brachylagus idahoensis (*n* = 4). Utah: Tooele Co.: Ibapah (UMNH:Mamm:12511), 3 mi SE Ibapah (UMNH:Mamm:7862). Nevada: Elko Co.: 10 mi N Wells (UMNH:Mamm:8820). White Pine Co.: Spring Valley, 8 km S, 5.6 km W Osceola (UMNH:Mamm:40907)

Sylvilagus audubonii (*n* = 13). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14630, 14631), Bagley Ranch, Callao (UMNH:Mamm:21765), Mayfield Ranch, 7 mi NE Trout Creek (UMNH:Mamm:21795), Snake Valley, junction Granite Creek Road and Snake Valley Road (UMNH:Mamm:37214). Millard Co.: Gandy Salt Marsh (BYU:Mamm:15062), 6 mi N Delta (UMNH:Mamm:24475), 6 mi NW Desert Experimental Range Station (UMNH:Mamm:24474). Tooele Co.: 2 mi W Willow Spring (UMNH:Mamm:21785), Clifton Flat, 5 mi S Gold Hill (UMNH:Mamm:21797), 6 mi S Ibapah (UMNH:Mamm:21787). Nevada, White Pine Co.: 0.4 km S, 5.2 km W Baker (BYU:Mamm:38296), Snake Creek, Snake Creek Range (UNR:Mamm:705).

Lepus californicus (*n* = 9). Utah: Juab Co.: Fish Springs National Wildlife Refuge (BYU:Mamm:14629), Boyd's Ranch, near Deep Creek Mountains (UMNH:Mamm:7753), 6 mi SW Eureka (MSB:Mamm:102737). Millard Co.: flats E of House Range, Springs Road (UMNH:Mamm:45998). Tooele Co.: 6 mi S Ibapah (BYU:Mamm:11748), 2.5 km N, 5.0 km W Gold Hill

(BYU:Mamm:37062). Nevada: White Pine Co.: 7.3 km W Baker (BYU:Mamm:38293), Snake Range, Willard Creek, 6.8 km S, 1.6 km E Osceola (UMNH:Mamm:40909), Duck Creek Valley (MSB:Mamm:107364).